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(56) References cited:
EP-A- 0 219 829 EP-A- 0 287 313

- **HETEROCYCLES, vol. 21, no. 1, 1984, pages 75-90; MORIO IKEHARA:
"2'-substituted2'-deoxypurinenucleotides
their conformation and properties"**
- **IDEAM**
- **CHEMICAL ABSTRACTS, vol. 108, no. 1, 4th January 1988, page 14, abstract no.199v,
Columbus, Ohio, US; V.E. MARQUEZ et al.:
"2',3'-Dideoxy-2'-fluoro-ara-A. An acid-stable
purine nucleoside active against human
immunodeficiency virus(HIV)", & **BIOCHEM.
PHARMACOL. 1987, 36(17), 2719-22****
- **CHEMICAL ABSTRACTS, vol. 109, no. 5, 1st August 1988, page 15, abstract no.31545e,
Columbus, Ohio, US; R. PAUWELS et al.:
"Investigations of the anti-HIVactivity of
2',3'-dideoxyadenosine analogs with
modifications in either thepentose of purine
moiety. Potent and selective anti-HIV activity of
2,6-diaminopurine 2',3'-dideoxyriboside", &
BIOCHEM. PHARMACOL. 1988,37(7), 1317-25**
- **JOURNAL OF BIOCHEMISTRY, vol. 87, 1980,
pages 825-830; A. ISHIHAMA et al.:
"2'-Deoxy-2'-azidoadenosine triphosphate and
2'-deoxy-2'-fluoroadenosinetriphosphate as
substrates and inhibitors for Escherichia coli
DNA-dependentRNA polymerase"**

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Description

The present invention relates to chemical compounds having anti-infectious activity, to methods for their preparation, to compositions containing them and their use particularly in the treatment of parasitic and viral diseases. More particularly the invention relates to 2'-deoxy-2'-fluoro-ribonucleosides and derivatives thereof.

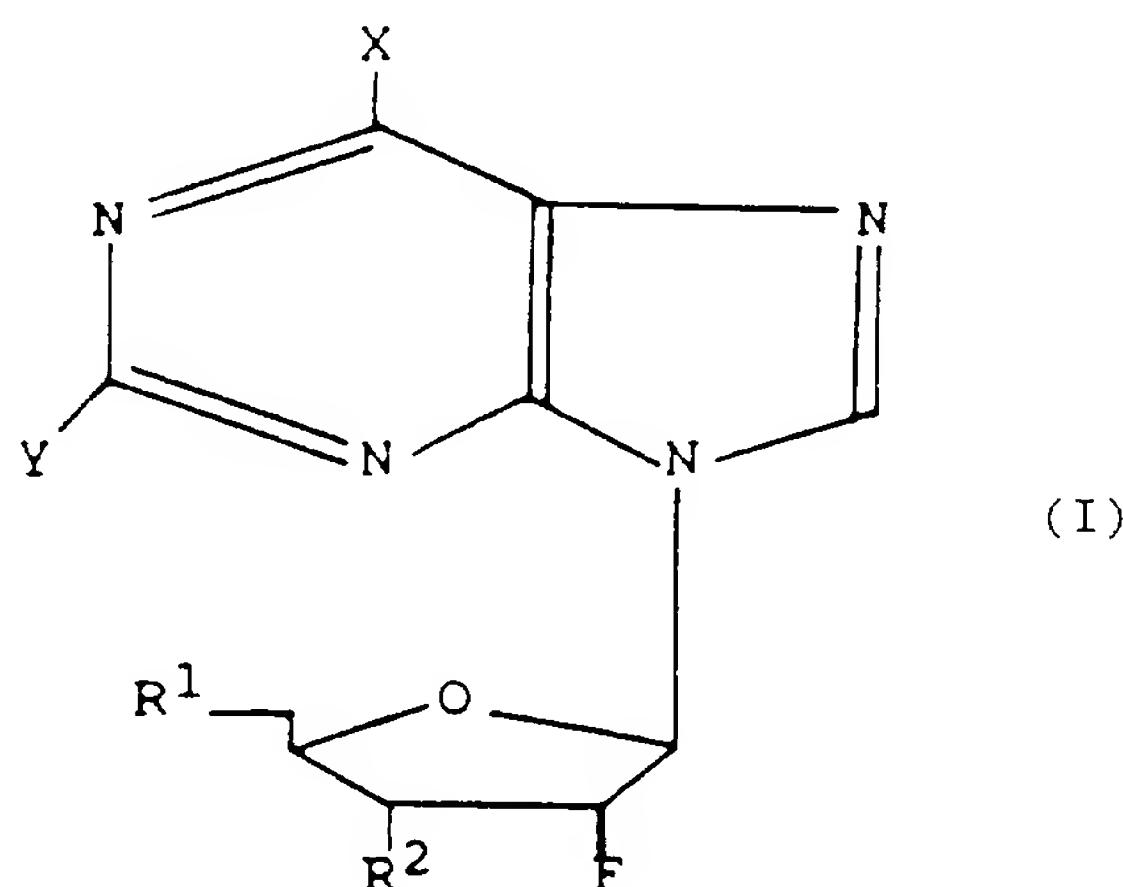
In the field of antiviral chemotherapy, few drugs exist which effectively combat the virus *per se*, owing to the difficulty of attacking the virus while leaving uninfected host cells unimpaired. It has recently been established that certain stages in the virus life-cycle, which vary from species to species, are specified by the virus itself. However, owing to great similarity between viral and host functions, effective treatments have proved very difficult to identify.

One group of viral pathogens which have afflicted man since ancient times and have been responsible for the deaths of many millions of people through the centuries are the influenza viruses. These viruses, particularly influenza A and B, remain one of the major causative agents of acute respiratory illness in the world to day.

Influenza viruses are members of the family of negative strand viruses Orthomyxoviridae. Another negative strand virus having important health implications is respiratory syncytial virus (RSV), which is a pneumovirus, a genus of the family Paramyxoviridae. RSV is a major cause of lower respiratory tract illness during infancy and childhood.

Fluorinated nucleosides have been previously proposed for the treatment of viral and parasitic diseases. For example, EP-A-0 287 313 discloses 2',3'-dideoxynucleosides, substituted with fluorine in the 2' position for use in the treatment of human immunodeficiency virus. EP-A-0 219 829 discloses 2'-deoxy-2'-fluoro- β -D-arabino-furanosyl nucleosides for use as antiparasitic agents, especially against leishmaniasis. Processes for the preparation of certain 2'-fluoro-2'-deoxy-ribonucleosides have been described by Uesugi *et al.*, (Nucleosides and Nucleotides 2, 373-, 1983) and Ikehara *et al.*, (Chem. Pharm. Bull. 29, 1034-, 1981).

We have now found that compounds of the formula (I) and their pharmaceutically acceptable salts are anti-infective agents, particularly against viruses. For example, the compounds of formula (I) are active against influenza virus, particularly influenza A and B and RSV infections, and certain protozoa, for example, Trichomonas vaginalis and Giardia lamblia. In formula (I):



Y is either H or NH₂;

X is a group -NR³R⁴ in which R³ and R⁴ may be the same or different and each represent hydrogen, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₃₋₇ cycloalkyl, each group optionally being substituted by one or more halogen, or X is a group Z-R⁵ in which Z is oxygen or sulphur and R⁵ has the same definition as R³, or X is halogen or hydrogen; and R¹ and R², which may be the same or different, each represent:

- a hydroxy group;
- a group -OCOR⁶H where R⁶ is a divalent group which is straight or branched C₁₋₆ alkylene, C₂₋₆ alkenylene or C₃₋₇ cycloalkylene, each being optionally substituted by one or more hydroxy groups;
- a group -OCO₂R⁷H where R⁷ is a covalent bond or is as defined for R⁶;
- a group -OCOR⁶-COOR⁸ where R⁶ is as defined above and R⁸ is selected from hydrogen, straight or branched chain C₁₋₆ alkyl or C₁₋₆ alkenyl each being optionally substituted by one or more hydroxy groups;
- a group -OCOR⁷-Z-Ar where R⁷ is as defined above, Z is either a covalent bond or oxygen and Ar is an aromatic ring, for example, a monocyclic aryl (e.g. phenyl) or a heteroaryl (e.g. pyridine), unsubstituted or substituted by one or more halogen, C₁₋₆ alkyl or C₁₋₆ alkoxy;
- a group -OR⁶H where R⁶ is as defined above;

- a group $-\text{OR}^6-\text{Z-Ar}$ where R^6 , Z and Ar are as defined above;
- a group $-\text{OCOCHR}^9\text{NR}^{10}\text{R}^{11}$ where R^{10} and R^{11} are as defined for R^8 , and R^9 is
 - hydrogen,
 - straight or branched chain C_{1-4} alkyl optionally substituted by one or more hydroxy, mercapto, C_{1-3} alkoxy or C_{1-3} alkylthio groups,
 - a group $-\text{R}^{12}-\text{A}$ where R^{12} is a C_{1-4} alkylene group optionally substituted by one or more hydroxy groups and A is a group $-\text{COOH}$, $-\text{CONH}_2$, $-\text{NH}_2$ or $-\text{NH}-\text{C}(\text{NH})\text{NH}_2$ or A is a 4- to 11- membered aromatic or non-aromatic cyclic or heterocyclic ring system containing 3 to 10 carbon atoms and 0, 1, 2 or 3 ring nitrogen atoms, the ring carbon atom and/or nitrogen atoms being optionally substituted by one or more hydroxy groups;
- a group $-\text{OCO}-\text{R}^{13}$ where R^{13} is a 4- to 7- membered heterocyclic ring containing 3 to 6 carbon atoms and 0, 1 or 2 nitrogen atoms, the ring carbon atoms and/or nitrogen atoms being optionally substituted by one or more hydroxy groups; or
- a mono-, di- or tri-phosphate ester group.

The present invention further includes compounds of formula (I) and their salts for use in medical therapy.

Preferred ester groups of the formula $-\text{OCOCHR}^9\text{NR}^{10}\text{R}^{11}$ and $-\text{OCO}-\text{R}^{13}$ are esters of naturally occurring α -amino acids included by the above definition. Especially preferred ester groups of the formula $-\text{OCOCHR}^9\text{NR}^{10}\text{P}^{11}$ are those wherein R^9 is C_{1-4} alkyl group and R^{10} and R^{11} are both hydrogen, for example, valine or alanine.

It will be appreciated that the compounds of Formula (I) may exist in various tautomeric forms, and that when X is a group $-\text{OH}$, it may also represent an oxo group. Compounds of formula (I) and their salts may also exist in α or β anomeric forms. The present invention therefore includes within its scope each of the individual α and β anomers of the compounds of formula (I) and mixtures thereof.

The compounds of formula (I) and their pharmaceutically acceptable salts will be hereinafter referred to as the compounds according to the invention. The term "active ingredient" as used hereafter, unless the context requires otherwise, refers to a compound according to the invention.

The activity of the active ingredients against influenza A and B has not previously been known and the present invention also provides pharmaceutical formulations comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof, especially useful for inhibiting the growth of influenza A or B in mammals and man. We therefore provide solid formulations comprising active ingredient and a solid carrier, or an inhalable formulation comprising active ingredient and inhalable fluid carrier.

The present invention further includes:

a) a method for the treatment or prophylaxis of a viral infection particularly influenza virus or RSV infection or a protozoal infection, for example, Trichomonas vaginalis or Giardia lamblia, in a host, for example a mammal including man, and mice which comprises treating said mammal with an effective non-toxic amount of a compound according to the invention.

b) use of a compound according to the invention in the manufacture of a medicament for the treatment or prophylaxis of an infection including a viral infection particularly influenza virus or RSV infection or a protozoal infection, for example, Trichomas vaginalis or Giardia lamblia.

Certain of the formula (I) compounds and their salts are new compounds and such new compounds and salts are a further aspect of the present invention. The new compounds are those, as defined above for formula (I) except for those in which R^1 and R^2 are each hydroxy or R^1 is a mono-, di- or tri-phosphate 5'-ester and R^2 is hydroxy or R^1 is hydroxy and R^2 is a mono-, di- or tri-phosphate 3'-ester and either (a) X is OH and Y is NH_2 or H or (b) X is NH_2 and Y is H .

Preferred compounds according to the invention include those wherein R^1 and R^2 are either both OH or R^1 is a monophosphate and R^2 is OH , Y is H or NH_2 and X is a group $-\text{NR}^3\text{R}^4$ in which R^3 and R^4 , which may be the same or different, represent H , C_{1-6} alkyl, provided R^3 and R^4 are not both hydrogen, or X is a group $\text{Z}-\text{R}^5$ in which Z is O or S and R^5 is C_{1-6} alkyl, or X is a halogen or hydrogen.

Particularly preferred compounds of formula (I) include those in which R^1 and R^2 are both OH , Y is NH_2 and X is H , NH_2 or $\text{Z}-\text{R}^5$ where Z is O and R^5 is C_{1-6} alkyl.

Examples of especially preferred compounds of formula (I) are:-

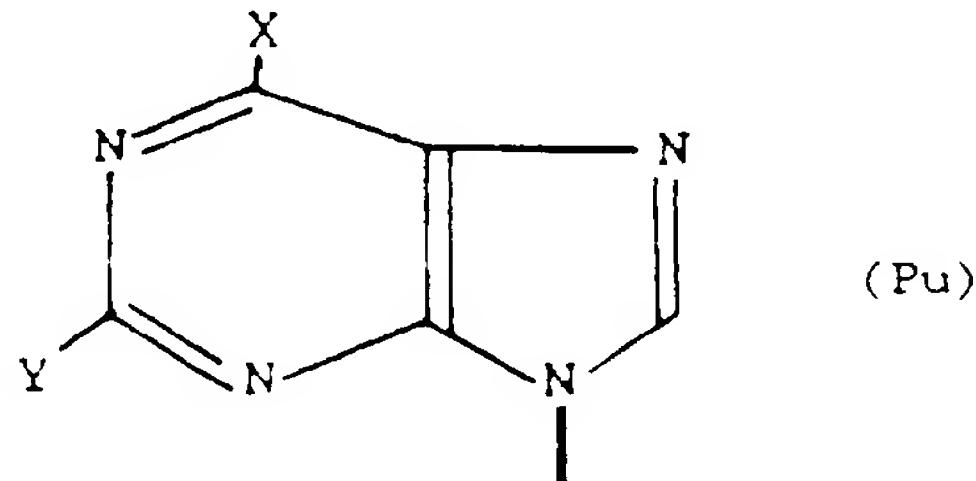
- (1) 2,6-Diamino-9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-9H-purine.
- (2) 2-Amino-9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-9H-purine.

(4) 2-Amino-9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-6-methoxy-9H-purine.

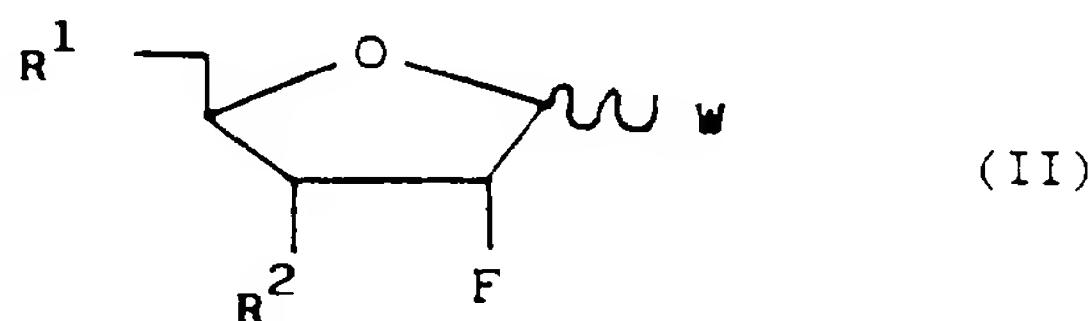
The present invention further includes a process for the preparation of the novel compounds of formula (I) and pharmaceutically acceptable salts thereof which comprises either:

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(A) reacting a purine base of formula PuH wherein Pu represents the purine residue:-



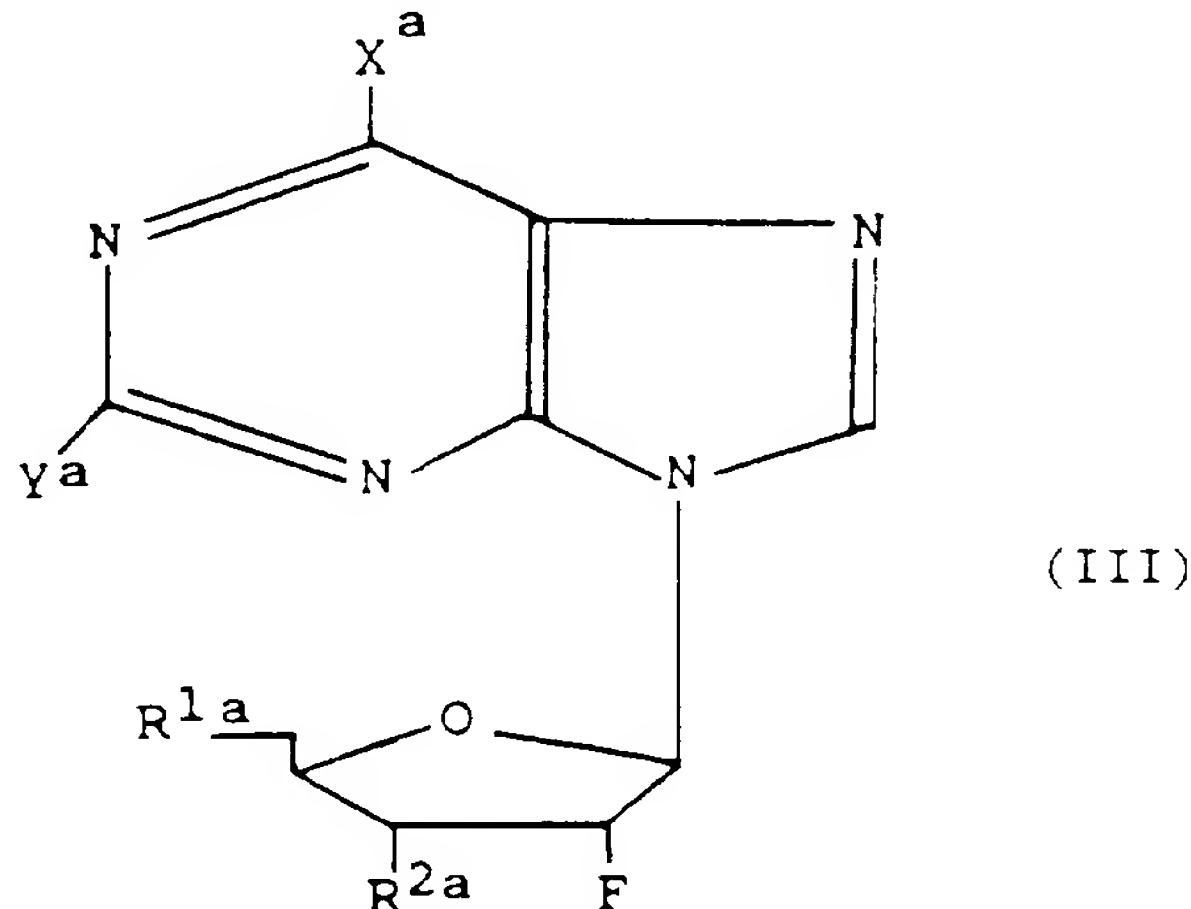
(wherein X and Y are as described above), or a salt thereof, with a compound of formula II)



wherein R¹ and R² are as hereinbefore defined, and W is either a phosphate ester or salt thereof or a purine or pyrimidine moiety (other than Pu) to form a compound of formula (I);

25

(B) reacting a compound of formula (III)



(wherein X^a, Y^a, R^{1a} and R^{2a} respectively represent the groups X, Y, R¹ and R² as defined above or a precursor, e.g. protected, form of such a group providing at least one of X, Y, R¹ and R² represents a precursor form) with an agent serving to convert the said precursor form of the group(s) to the desired group(s);

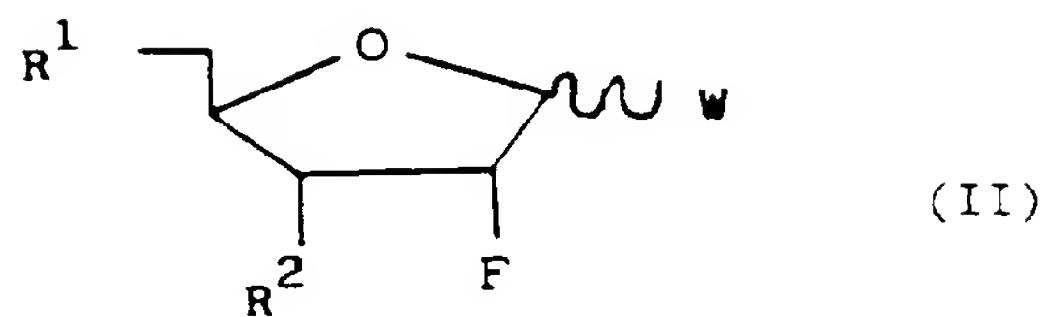
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(i) reacting a compound of formula (I) wherein at least one of R¹ and R² is a hydroxy group with an appropriate agent serving to convert the said hydroxy group to an alternative group represented by R¹ and/or R².

50

(ii) reacting a compound of formula (I) wherein R¹ and/or R² are not hydroxy with an agent serving to convert the said R¹ and/or R² group to a hydroxy group.

Process (A), which is particularly suitable for the preparation of compounds of formula (I) wherein R¹ and R² are both hydroxy and Y is H or NH₂, may be effected enzymatically by reacting the purine base of formula Pu wherein X and Y are as hereinbefore defined, or a salt thereof, with a compound of formula (II)



(wherein R¹ and R² are as hereinbefore defined and W is a phosphate ester or salt thereof or a purine or pyrimidine moiety (other than Pu)) in the presence of at least one phosphorylase enzyme such as purine nucleoside phosphorylase and thymidine phosphorylase and an inorganic phosphate or a salt thereof, or a transferase enzyme, for example, N-deoxyribosyl transferase. For the purposes of this reaction it is preferred that the purine or pyrimidine moiety be in the β -position and the phosphate ester or salt thereof in the α -position.

15 The appropriate purine base of formula PuH may be obtained commercially, for example, from Pacific Chemical Laboratories or Sigma Chemical Company or prepared by conventional methods well known to a skilled person or readily available from the chemical literature. For example, purine bases where the 2- substituent is amino or hydrogen and the 6-substituent is amino or methylthio may be obtained commercially. Purines where the 2-substituent is amino and the 6-substituent is a methylamino group may be prepared according to the method of Montgomery and Holum (J.A.C.S. 80:404, 1958). Purines in which the 2-substituent is amino and the 6-substituent is alkoxy, for example, methoxy, may be prepared by the method described by Balsiger and Montgomery (J. Org. Chem. 20 : 1573, 1960).

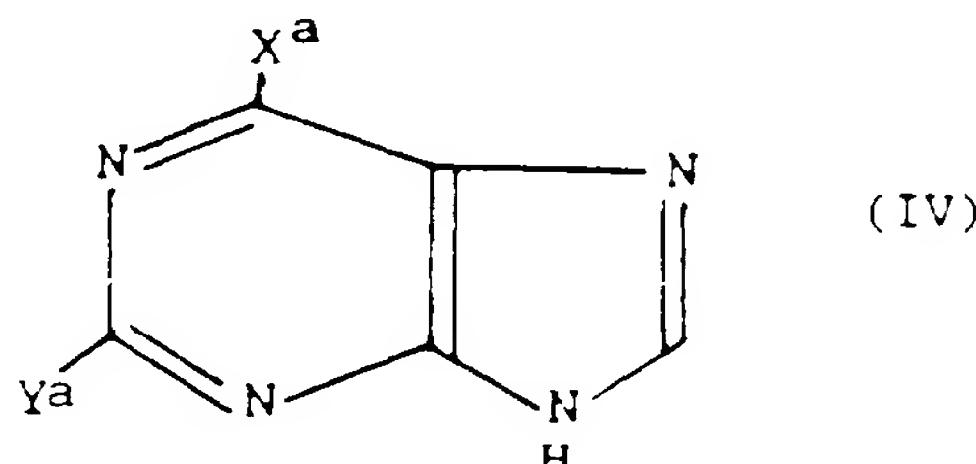
20 Compounds of formula (II) may be prepared by methods well known to a skilled person or readily available from the chemical literature. For example, 1-(2-deoxy-2-fluoro- β -D-ribofuranosyl)uracil may be prepared by the method described in Codington *et al.* (J. Org. Chem. 29 : 558, 1964). 9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)adenine may be prepared by the method described by S.Uesugi *et al* (Nucleosides and Nucleotides 2, 373-, 1983).

25 With regard to process (B), which is particularly suitable for the preparation of compounds of formula (I) from compounds of formula (III) wherein R^{1a} and R^{2a} are both hydroxy, the protected groups X^a, Y^a, R^{1a} R^{2a} of compounds of formula (III) may be protected with conventional hydroxy and amino protecting groups such as acyl groups, for example, alkanoyl such as benzoyl, or aroyl groups such as acetyl; aralkyl groups for example a benzyl group; or trialkylsilyl groups such as tert-butyldimethylsilyl, the particular type of protecting group employed being dependent on the nature of the group to be protected.

30 The protecting group can subsequently be removed by acid or base hydrolysis, hydrogenolysis or enzymatically. Acyl groups are typically removed by base hydrolysis and silyl groups by acid hydrolysis or fluoride ion. Aralkyl groups such as benzyl are advantageously removed by catalytic hydrogenolysis, typically in the presence of a catalyst such as palladium on charcoal or reductively, for example, with an alkali metal (e.g. sodium) in a suitable solvent such as liquid ammonia. It has been found that the use of a benzyl protecting group is particularly advantageous for the preparation of compounds of formula (I) wherein X is hydroxy or amino.

35 For the preparation of a compound of formula (I) in which R¹ and/or R² represents a group OCOCHR⁹NR¹⁰R¹¹, in accordance with process (B) the groups R^{1a} and/or R^{2a} may be protected with, for example, 9-fluorenylmethoxy carbonyl (FMOC), tert-butyloxycarbonyl (tBOC) or carbobenzyloxy (CBZ) and may be deprotected by conventional means to give the amino acid CHR⁹NR¹⁰R¹¹.

40 The starting compound of formula (III) may conveniently be prepared by reacting an appropriate purine base of formula (IV)



50 wherein X^a and Y^a are as hereinbefore defined; or a salt thereof, with a pyrimidine nucleoside containing the appropriate sugar residue, for example, 1-(2-deoxy-2-fluoro- β -D-ribofuranosyl)uracil in the presence of one or more phosphorylase enzymes, for example, purine nucleoside phosphorylase and thymidine phosphorylase, or a transferase enzyme, for example N-deoxyribosyl transferase.

55 Alternatively a starting compound of formula (III) above can be prepared chemically by reacting a compound of formula (IV) wherein X^a and Y^a are as hereinbefore defined, or a silylated derivative thereof, or a salt thereof, with a compound of formula (II) wherein R¹ and R² are as hereinbefore defined or other protected forms of hydroxy and W is

a suitable leaving group, for example, a halogen atom, such as chlorine, an acyloxy group such as acetoxy, an alkoxy group such as methoxy, in the presence of a catalyst such as tin (IV) chloride or trimethylsilyl triflate in a suitable solvent such as acetonitrile.

Purine bases of formula (IV) may be prepared from a corresponding purine base wherein the 6-substituent is a suitable leaving group, for example, a halogen atom, such as chlorine, by nucleophilic displacement of said group. Thus a purine base of formula (IV) wherein X^a is benzyloxy may be prepared for example, by treatment of the corresponding 6-chloro purine with an alcohol such as benzyl alcohol in the presence of a base, for example, sodium hydride in a suitable solvent such as tetrahydrofuran (THF). Purine bases of formula (IV) wherein X^a is benzylamino may be prepared by treatment of the corresponding 6-chloropurine with an amine, for example benzylamine in the presence of a base, for example, an amine such as triethylamine in a suitable solvent such as methanol. Such purine bases used as the above starting materials may be obtained commercially (Aldrich Chemical Company) or prepared by methods well known to a skilled person or readily available from the chemical literature.

Pyrimidine nucleosides employed in the above preparation of the compounds of formula (III) may be prepared by methods well known to a skilled man or readily available from the chemical literature. For example, 1-(2-deoxy-2-fluoro- β -D-ribofuranosyl)pyrimidines, such as 1-(2-deoxy-2-fluoro- β -D-ribofuranosyl)uracil, may be prepared by the method described in Codington *et al* (J.Org. Chem. 29 : 558, 1964).

It will be appreciated that compounds of formula (III) may represent compounds according to the invention thus, for example, X^a may represent $-NR^3R^4$ where R^3 and R^4 are as hereinbefore defined. Such compounds may be treated with a deaminase enzyme such as adenosine deaminase and converted to a compound of formula (I) where X is hydroxy.

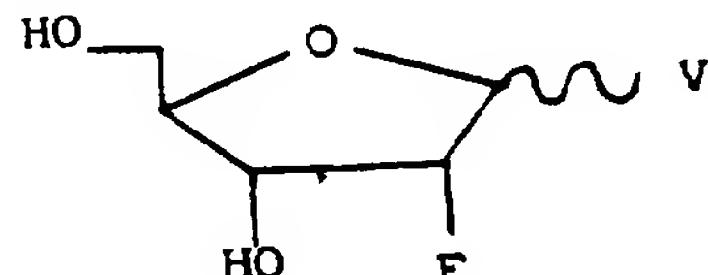
A compound of formula (I), wherein R^1 and/or R^2 are hydroxy may be converted into a corresponding pharmaceutically acceptable ester of formula (I) as hereinbefore defined, by reaction with an appropriate esterifying agent according to methods known in the art, for example, by the method described by Martin *et al*, J.Pharm.Sci. (1987) 76, 180-. Thus, for the preparation of appropriate carboxylic acid esters as defined above the former compound of formula (I) may be reacted with an acid anhydride or acid halide, for example, chloride corresponding to a carboxylic acid of the formula, $QCOOH$ where Q is a group $-R^6H$, $-OR^7H$, $-R^6COOR^8$, $-R^7-Z-Ar$, $-CHR^9NR^{10}R^{11}$ or $-R^{13}$ where R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{13} , Z and Ar are as defined above, in the presence of a suitable base such as triethylamine. Alternatively, the acid $QCOOH$ may be reacted with a compound of formula (I) wherein R^1 and/or R^2 are hydroxy in the presence of a coupling agent such as dicyclohexylcarbodiimide.

The above starting material of formula (II) wherein R^1 and/or R^2 are hydroxy may be reacted with an esterifying agent and the resulting compound reacted with the purine base of formula PuH in accordance with process (A).

Compounds according to the invention when R^1 and/or R^2 are mono-, di- or tri- phosphate esters may be prepared from compounds of formula (I) when R^1 and/or R^2 are hydroxy by successive phosphorylation via the mono-, di- and tri- phosphate derivatives by conventional chemical means or by enzymatic means, for example using a nucleoside kinase or phosphotransferase in the presence of a nucleotide triphosphate, for example ATP.

A compound of formula (I) wherein R^1 and/or R^2 are hydroxy may be converted into a corresponding pharmaceutically acceptable ether of formula (I), as hereinbefore defined, by reaction with an appropriate alkylating agent in a conventional manner, for example, a compound of formula Q^1-Hal wherein Q^1 is a group $-R^6H$ or $-R^6-Z-Ar$ where R^6 and Ar are as hereinbefore defined and Hal is a halogen, e.g. iodine.

Alternatively, such an alkylating agent can be reacted with a sugar of formula



(wherein V is an alkoxy group such as methoxy, or an acyloxy group, such as acetoxy) to obtain a corresponding 3', 5'- or 3',5'-di-ether, (Bessodes M *et al*, Synthesis (1988) 560) and condensing the resulting ether with a suitable purine base of formula PuH to form a nucleoside which is then deprotected in accordance with process (B) above.

Where the product obtained from the first step of the above process is a mono-ether, for example a 5'-ether, the other hydroxy group may be reacted with a carboxylic acid or derivative thereof, e.g. an acid anhydride or acid halide, e.g. chloride corresponding to a carboxylic acid of formula $QCOOH$ where Q is as defined above, to obtain a mono-ether-mono-ester sugar, e.g. a 3'-ester-5'-ether sugar. This sugar can be condensed with a suitable purine to form a nucleoside as mentioned above, or by reference to Codington J.F. *et al*, Carbohyd. Res. (1966) 1,455.

Compounds according to the invention may also be obtained from compounds of the formula I' , where I' is a compound corresponding to the compound of formula (I) in which the groups R^1 and R^2 are replaced by other protecting groups. These other protecting groups may be removed or reacted to produce the groups R^1 and R^2 as defined above.

Compounds according to the invention, when produced by the route described above, can be used to obtain compounds of the invention when R^1 and/or R^2 are hydroxy by deprotecting the ether and/or ester groups.

Depending on the process conditions and the starting materials, the end product of the formula (I) is obtained either as the free base or as a salt. Both the free base and the salts of the end products are included within the scope of the invention. Thus, basic, neutral or mixed salts may be obtained as well as hemi-, mono-, sesqui- or poly-hydrates. Acid addition salts of the new compounds may in a manner known per se be transformed into free base using basic agents such as alkali or by ion exchange. The free bases obtained may also form salts with organic or inorganic acids.

5 Salts according to the invention which may be conveniently used in therapy include pharmaceutically acceptable base salts, eg derived from an appropriate base, such as alkali metal (e.g. sodium), alkaline earth metal (e.g. magnesium) salts, ammonium and NX_4 (wherein X is C_{1-4} alkyl) salts.

10 The present invention further includes the novel intermediates:

2-amino-6-benzylamino-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-9H-purine,
2-amino-6-benzylxy-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-9H-purine, and
2-amino-6-benzylthio-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-9H-purine.

15 The compounds according to the invention may be administered to recipients such as mammals including humans by any route appropriate to the condition to be treated, suitable routes including oral, pulmonic, rectal, nasal, topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural). It will be appreciated that the preferred route may vary with, for example, the condition of the recipient.

20 The amount required of the individual active ingredient for the treatment of a viral infection including influenza and RSV infections will depend upon a number of factors including the severity of the condition to be treated and the identity of the recipient and will ultimately be at the discretion of the attendant physician. In general, a suitable, effective dose will be in the range 0.1 to 100 mg per kilogram body weight of recipient per day, preferably in the range 5 to 30 mg per kilogram body weight per day and most preferably in the range 10 to 20 mg per kilogram body weight per day; an optimum dose is about 15 mg per kilogram body weight per day (unless otherwise indicated all weights of active ingredient are calculated as the parent compound; for salts and esters thereof the figures would be increased proportionately.) The 25 effective dose may optionally be presented as two, three, four or more sub-doses administered at appropriate intervals throughout the day. These sub-doses may be administered in unit dosage forms, for example, containing 1 to 2000 mg preferably 20 to 500 mg and most preferably 100 to 400 mg of active ingredient per unit dosage form.

30 The compounds according to the present invention may be administered alone or in combination with other therapeutic agents, for example, with amantidine or with ribavirin, which are known anti-influenza agents, or an analgesic, for example, codeine, paracetamol, aspirin, ibuprofen or an anti-inflammatory agent, such as, indomethacin, mefenamic acid, naproxen, or ibuprofen or any other agents which when in combination with a compound according to the invention provide a beneficial therapeutic effect.

35 While it is possible for the compounds to be administered alone it is preferable to present them as pharmaceutical formulations. The formulations according to the present invention comprise at least one active ingredient, as above defined, together with one or more pharmaceutically acceptable carriers therefor and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipients thereof.

40 The formulations include those suitable for oral, pulmonic, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

45 Formulations according to the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, or paste or may be contained within liposomes.

50 A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in varying proportions to provide the desired release profile.

55 A capsule may be made by filling a loose or compressed powder on an appropriate filling machine, optionally with

one or more additives. Examples of suitable additives include binders such as povidone, gelatin, lubricants, inert diluents, disintegrants as for tablets. Capsules may also be formulated to contain pellets or discrete sub-units to provide slow or controlled release of the outline ingredient. This can be achieved by extruding and spheronising a wet mixture of the drug plus an extrusion aid (e.g. microcrystalline cellulose) plus a diluent such as lactose. The spheroids thus produced can be coated with a semi-permeable membrane (e.g. ethyl cellulose, Eudragit WE30D) to produce sustained release properties.

For topical administration the formulations are preferably applied as an ointment or cream containing the active ingredient in an amount of, for example, 0.075 to 20% w/w, preferably 0.2 to 15% w/w and most preferably 0.5 to 10% w/w. When formulated in an ointment, the active ingredients may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with an oil-in-water cream base or as in a water in oil base.

If desired, the aqueous phase of the cream base may include, for example, at least 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active ingredient through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulphoxide and related analogues.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While this phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and/or fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glycerol mono-stearate and sodium lauryl sulphate.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the active compound in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isooctyl adipate, isooctyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oil can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active ingredient. The active ingredient is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% particularly about 1.5% w/w.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or higher fatty alcohol (e.g. hard wax European Pharmacopoeia) or triglycerides and saturated fatty acids (e.g. Witepsol).

Formulations suitable for nasal administration wherein the carrier is a solid include a coarse powder having a particle size for example in the range 20 to 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid, for administration as for example a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Suitable formulations for administration by inhalation include fine particle dusts or mists which may be generated by means of various types of metered dose pressurised aerosols, nebulizers or insufflators.

For pulmonary administration via the mouth, the particle size of the powder or droplets is typically in the range 0.5 - 10 μ m, preferably 1 - 5 μ m, to ensure delivery into the bronchial tree. For nasal administration, a particle size in the range 10 - 500 μ m is preferred to ensure retention in the nasal cavity.

Metered dose inhalers are pressurised aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in liquefied propellant. During use these devices discharge the formulation through a valve adapted to deliver a metered volume, typically from 10 - 150 μ l, to produce a fine particle spray containing the active

ingredient. Suitable propellants include propane and butane, certain chlorofluorocarbon compounds, commonly referred to as "CFS's", for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, or mixtures thereof. The formulation may additionally contain co-solvents, for example ethanol, surfactants such as oleic acid or sorbitan trioleate, antioxidants and/or suitable flavouring agents.

5 Nebulizers are commercially available devices that transform solutions or suspensions of the active ingredient into an aerosol therapeutic mist either by means of acceleration of a compressed gas through a narrow venturi orifice, typically air or oxygen, or by means of ultrasonic agitation. Suitable formulations for use in nebulizers consist of the active ingredient in a liquid carrier and comprising up to 40%w/w of the formulation, preferably less than 20%w/w. The carrier is typically water or a dilute aqueous alcoholic solution, preferably made isotonic with body fluids by the addition of, for
10 example, sodium chloride. Optional additives include preservatives if the formulation is not prepared sterile, for example methylhydroxybenzoate, antioxidants, flavouring agents, volatile oils, buffering agents and surfactants.

15 Suitable formulations for administration by insufflation include finely comminuted powders which may be delivered by means of an insufflator or taken into the nasal cavity in the manner of a snuff. In the insufflator, the powder is contained in capsules or cartridges, typically made of gelatin or plastic, which are either pierced or opened in-situ and the powder either presented to air drawn through the device upon inhalation or alternatively delivered by means of a manually operated pump. The powder employed in the insufflator consists either solely of the active ingredient or of a powder blend comprising the active ingredient, a suitable powder diluent such as lactose, and an optional surfactant. The active ingredient typically comprises from 0.1 - 100%w/w of the formulation.

20 Pressurised aerosol formulations for inhalation are preferably arranged so that each metered dose contains from 0.05 to 5 mg of a compound of the invention. Similarly, powder formulations for insufflations are so arranged that each unit dose contains from 0.5 to 50 mg. Solution or suspension formulations for nebulisation are arranged as to deliver doses between 1 and 1500 mg. The compounds according to the invention or formulations thereof may be administered by these devices once or several times daily, with one or several doses, for example three or four, being given on each occasion.

25 Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

30 Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Injection solutions and suspensions 35 may be prepared extemporaneously from sterile powders, granules and tablets of the kind previously described.

35 Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as herein above recited, or an appropriate fraction thereof, of an active ingredient.

40 It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

The following Examples are provided by way of illustration of the present invention and should in no way be construed as limitation thereof.

Example 1

Preparation of 9-(2-Deoxy-3,5-di-O propionyl-2-fluoro- β -D-ribofuranosyl)guanine

To a solution of 9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)guanine (170mg, 0.6mmol), DMAP (4-dimethylaminopyridine) (8mg) and triethylamine (0.41 ml, 5 eq.) in DMF (*N,N*-dimethylformamide) (4ml) at room temperature was added propionic anhydride (0.16ml, 2.1 eq) with stirring. After 20 hours, methanol (2ml) was added. One hour later the mixture was evaporated in vacuo and the residue was chromatographed over SiO_2 using the flash technique. Elution was with $\text{CHCl}_3/\text{MeOH}$ (10:1), then (6:1), affording the title compound as a white solid.
mp. 198-200°C.

Anal. Calc. for 0.2. hydrate:	C, 47.93;	H, 5.13;	N, 17.47
Found:	C, 48.22;	H, 4.94;	N, 17.0

Example 2Preparation of 9-(2-Deoxy-3,5-di-O-acetyl-2-fluoro- β -D-ribofuranosyl)guanine, and 9-(2-deoxy-2-fluoro-3,5-di-O-acetyl- β -D-ribofuranosyl)-2-N-acetyl guanine

5 To a solution of 9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)guanine (160mg, 0.56 mmol), DMAP (8mg) and triethylamine (400 μ l 5eq.) in DMF (4ml) was added acetic anhydride (0.16ml, 2.5eq) with stirring at room temperature. After 22 hours the yellow solution was evaporated in vacuo, co-evaporated with ethanol and toluene and chromatographed over SiO₂ using the flash technique and eluting with CHCl₃/MeOH (6:1). The first eluted component was evaporated, and co-evaporated with toluene to afford the triacetyl derivative as a white foam.

Anal. Calc. for 0.1 toluate:	C, 47.69;	H, 4.51;	N, 16.65
Found:	C, 47.47;	H, 4.37;	N, 16.82

15 Collection and evaporation of fractions containing the second eluted compound gave the di-acetyl derivative as a white solid.
mp. 232-234°C (dec.)

Anal. Calc. for 0.25 ethanolate:	C, 45.73;	H, 4.63;	N, 18.39
	C, 46.02;	H, 4.34;	N, 18.19

Example 3Preparation of 9-(2-Deoxy-2-fluoro-3-O-pivaloyl- β -D-ribofuranosyl)-guanine, and 9-(2-Deoxy-2-fluoro-3,5-di-O-pivaloyl- β -D-ribofuranosyl)guanine

25 To a solution of 9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)guanine (200mg, 0.70 mmol), DMAP (10mg) and triethylamine (0.5ml) in DMF (6ml) at room temperature was added trimethylacetic anhydride (160 μ l, 1.1eq) and the solution stirred for 24 hours. A further 80 μ l trimethylacetic anhydride was then added and stirring continued for five days. The reaction was quenched with methanol (1ml), evaporated under reduced pressure to a white solid, and chromatographed over SiO₂ using the flash technique, eluting with CHCl₃/MeOH (15:1), then (10:1), (6:1), and finally (4:1).

30 The third eluted UV absorbing fraction was the 3',5'-bis pivalate ester as a waxy solid after trituration with ether.
mp. 250-252°C (dec.).

Anal. C ₂₀ H ₂₈ FN ₅ O ₆ ·0.5H ₂ O requires:	C, 51.94;	H, 6.32;	N 15.15
Found:	C, 51.99;	H, 6.23;	N, 14.83

35 The fourth eluted UV absorbing fraction was the 3'-pivalate ester as a white powder. mp. gradually darkens from 260-320°C.

Anal. C ₁₅ H ₂₀ FN ₅ O ₅ 0.7H ₂ O requires:	C, 47.17;	H, 5.65;	N, 18.34
Found:	C, 47.10;	H, 5.36;	N, 17.93

Example 4Preparation of 9-(2-Deoxy-2-fluoro-3,5-di-O-pivaloyl- β -D-ribofuranosyl)adenine, 9-(2-Deoxy-2-fluoro-3-O-pivaloyl- β -D-ribofuranosyl)adenine and 9-(2-Deoxy-2-fluoro-5-O-pivaloyl- β -D-ribofuranosyl)adenine

50 To a solution of 9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)adenine (250mg, 0.93 mmol) DMAP (10mg) and triethylamine (0.65ml) in dry DMF (7ml) was added trimethylacetic anhydride (226 μ l 1.2eq) at room temperature with stirring. After 24 hours a further 60 μ l trimethylacetic anhydride was added, followed 24 hours later by a further 20 μ l. After one further day the reaction was quenched with MeOH, evaporated in vacuo, co-evaporated with ethanol and purified by flash chromatography over SiO₂. Elution was with CHCl₃/MeOH (18:1), (15:1) and finally (10:1). The first eluted UV absorbing fraction was the 3'5'-bis ester as a white solid after trituration with ether.
mp. 157-158°C

Anal. C ₂₀ H ₂₈ FN ₅ O ₅ .0.3H ₂ O requires:	C, 54.24;	H, 6.51;	N, 15.82
Found:	C, 54.40;	H, 6.33;	N, 15.48

5 The second eluted UV absorbing fraction was the 3'-pivalate ester as a white solid.
mp. 204-205°C.

Anal. for C ₁₅ H ₂₀ FN ₅ O ₄ .0.3H ₂ O requires:	C, 50.22;	H, 5.79;	N, 19.53
Found:	C, 50.25;	H, 5.58;	N, 19.31

10 The third eluted UV absorbing fraction was the 5'-pivalate ester as a white foam.
mp. 130-134°C

Anal. C ₁₅ H ₂₀ FN ₅ O ₄ .0.2H ₂ O requires:	C, 50.46;	H, 5.76;	N, 19.62
Found:	C, 50.57;	H, 5.65;	N, 19.45

Example 5

20 Preparation of 9(2-Deoxy-2-fluoro-3,5-di-O-valeryl-β-D-ribofuranosyl)adenine, 9
(2-Deoxy-2-fluoro-3-O-valeryl-β-D-ribofuranosyl)adenine and 9(2-Deoxy-2-fluoro-5-O-valeryl-β-D-ribofuranosyl)
adenine

25 To a solution of 9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)adenine (300mg, 1.11mmol) DMAP (10mg) and triethylamine
(0.9ml) in dry DMF (8ml) was added valeric anhydride (263μl 1.2 eq) at room temperature with stirring. At 24 and 48
hours a further 30μl valeric anhydride was added. One day after the final addition the mixture was quenched with MeOH,
and then worked up and chromatographed as for the preparation of the pivalate esters. The first eluted UV absorbing
component was the 3',5'-bis ester, which crystallised as white needles upon trituration with ether.
mp. 96-98°C.

30

Anal. C ₂₀ H ₂₈ FN ₅ O ₅ requires:	C, 54.91;	H, 6.45;	N, 16.01
Found:	C, 54.93;	H, 6.49;	N, 15.89

35 The second eluted UV absorbing component was the 3'-valerate ester.
mp. 182-183°C.

Anal. C ₁₅ H ₂₀ FN ₅ O ₄ requires:	C, 50.98;	H, 5.71;	N, 19.82
Found:	C, 50.91;	H, 5.82;	N, 19.46

40 The third eluted UV absorbing component was the 5'-valerate ester, which crystallised upon trituration with ether.
mp. 115-117°C

45

Anal. C ₁₅ H ₂₀ FN ₅ O ₄ requires:	C, 50.98;	H, 5.71;	N, 19.82
Found	C, 50.76;	H, 5.81;	N, 19.44

Example 6

50 2,6-Diamino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine

55 2,6-Diaminopurine (Pacific Chemical Laboratories, 0.8 g, 4.8 mmoles) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)
uracil (0.4 g, 1.6 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were
suspended in 50 ml of 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. Thy-
midine phosphorylase (3,850 I.U.) and purine nucleoside phosphorylase (3,760 I.U.) (T.A. Krenitsky et al., Biochemistry
20:3615, 1981 and U.S. Patent 4,381,344) were added and the suspension stirred at 37°C. On day 12, the reaction was
filtered. The filtrate was applied to a 1.5 x 15 cm column of anion exchange resin (Bio-Rad AG1X2-hydroxide form).
After washing the column with water/methanol (7/3), the product was eluted with water/methanol (1/1). Product contain-

ing fractions were combined and the solvent removed under vacuum. The residue was dissolved in water and lyophilized to obtain title compound that analyzed as a 1.2 hydrate.

mp. 124°C UV λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1N HCl, 291 (10.2), 252 (11.8); pH 7, 278.5 (10.3), 255 (9.69); 0.1N NaOH, 279 (10.6), 255 (9.69).

5

Anal. Calcd. for $C_{10}H_{13}FN_6O_{31} \cdot 2 H_2O$:				
Calcd.:	C, 39.27;	H, 5.07;	N, 27.48;	F, 6.21.
Found:	C, 39.22;	H, 5.09;	N, 27.39;	F, 6.45.

10

$^1\text{H-NMR}$ (80mHz, $\text{Me}_2\text{SO-d}6$): δ 7.94 (s, 1H, H-8); 6.74 and 5.79 (2 bs, 4H, 2-NH2 and 6-NH2); 6.04 (dd, 1H, H-1', $JF,1' = 16.5$ Hz, $J = 3.4$ Hz); 5.64 (m, 1.5H, 0.5 (H-2') and 3'-OH); 5.25 (t, 1H, 5'-OH, $J = 5.5$ Hz); 4.98 (m, 0.5H, 0.5 (H-2')); 4.41 (m, 1H, H-3'); 3.93 (m, 1H, H-4'); 3.65 (m, 2H, $H_{\alpha}-5'$ and $H_{\beta}-5'$).

15 Example 79-(2-Deoxy-2-fluoro- β -D-ribofuranosyl)adenine

Adenine (Mann Research Laboratories, Inc., 0.8 g, 5.9 mmoles) and 1-(2-deoxy-2-fluoro- β -D-ribofuranosyl)uracil (0.4 g; 1.6 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were suspended in 20 ml of 10 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. Thymidine phosphorylase (2,400 I.U.) and purine nucleoside phosphorylase (3,900 I.U.) (T.A. Krenitsky et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,344) were added and the suspension stirred at 37°C. On day 6, the reaction was diluted to 100 ml with 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. On day 17, the suspension was filtered and the filtrate was evaporated. The residue was suspended in warm water. The suspension was filtered and the filtrate applied to a 1.5 x 12 cm column of anion exchange resin (Bio-Rad AGIX2-hydroxide form). The product was eluted with water. Product containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in water and lyophilized to obtain the title compound that analyzed as a 0.6 hydrate.

mp. 225-227°C nm (dec.)

UV λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1N HCl, 257 (14.6); 0.1N NaOH, 260 (14.9).

Anal. Calcd. for $C_{10}H_{12}FN_5O_3 \cdot 0.6 H_2O$:				
Calcd.:	C, 42.89;	H, 4.57;	N, 25.01;	F, 6.78.
Found:	C, 42.94;	H, 4.76;	N, 24.98;	F, 6.89.

35

$^1\text{H-NMR}$ (250 mHz, $\text{Me}_2\text{SO-d}6$): δ 8.35 and 8.14 (2s, 2H, H-8 and H-2); 7.36 (s, 2H, 6-NH2); 6.21 (dd, 1H, H-1', $JF,1' = 16.8$ Hz, $J = 3.0$ Hz); 5.71(d, 1H, 3'-OH, $J = 6.0$ Hz); 5.42 (ddd, 1H, H-2', $JF,2' = 53.0$ Hz, $J1',2' = 3.0$ Hz, $J2',3' = 4.5$ Hz); 5.25 (t, 1H, 5'-OH, $J = 5.6$ Hz); 4.47 (m, 1H, H-3'); 3.97 (m, 1H, H-4'); 3.74 (m, 1H, $H_{\alpha}-5'$); 3.56 (m, 1H, $H_{\beta}-5'$).

40

Example 82-Amino-9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-9H-purine

2-Aminopurine (Vega Biochemicals, 0.4 g, 3.0 mmoles) and 1-(2-deoxy-2-fluoro- β -D-ribofuranosyl)uracil (0.2 g, 0.71 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were suspended in 35 ml of 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. Thymidine phosphorylase (1,930 I.U.) and purine nucleoside phosphorylase (1,880 I.U.) (T.A. Krenitsky et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,344) were added and the suspension stirred at 37°C. On day 24, the reaction was diluted to 200 ml with water and 1,390 I.U. of thymidine phosphorylase, and, 1,880 I.U. of purine nucleoside phosphorylase were added. On day 35, the suspension was evaporated. The residue was dissolved in water/methanol (7/3) and applied to a 1.5 x 15 cm column of anion exchange resin (Bio-Rad AGIX2-hydroxide form). The product was eluted with water/methanol (7/3). The product-containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in water and lyophilized to obtain title compound that analyzed as a 0.6 hydrate.

mp. 151-153°C.

UV λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1N HCl, 313 (4.00), 240-245; pH 7, 304 (7.00), 243; 0.1N NaOH, 304 (7.30), 243.

5

Anal. Calcd. for C ₁₀ H ₁₂ FN ₅ O ₃ .0.6 H ₂ O:				
Calcd.:	C, 42.89;	H, 4.75;	N, 25.01;	F, 6.78.
Found:	C, 43.02;	H, 4.71;	N, 25.12;	F, 6.58

¹⁰ ¹H-NMR (80 mHz, Me₂SO-d₆): δ 8.62 and 8.31 (2s, 2H, H-8 and H-6); 6.62 (bs, 2H, 2-NH₂); 6.16 (dd, 1H, H-1', JF,1'=16.7 Hz, J = 2.7 Hz); 5.71 (m, 1.5H, 3'-OH and 0.5 (H-2')); 5.12 (m, 1.5H, 5'-OH and 0.5 (H-2')); 4.40 (m, 1H, H-3'); 3.97 (m, 1H, H-4'); 3.66 (m, 2H, H_α-5' and H_β-5').

10

Example 9

2-Amino-6-cyclopropylamino-9H-purine-hydrochloride

¹⁵ A solution of 2-amino-6-chloro-purine (Aldrich Chemical Company 4.66g, 27.5mmoles) and cyclopropylamine (Aldrich Chemical Company, 12.5g, 220mmoles) in MeOH (100ml) was heated at 50°C for 18 hours. Then 2-methoxyethanol (50ml) was added, and the reaction was heated at 70°C for an additional 6 hours. After cooling, a small amount of unreacted starting material was filtered off, and the filtrate was evaporated and purified on a silica gel column with CHCl₃: 5% to 10% MeOH. The product was then recrystallized twice from MeOH and once from EtOH as a hydrochloride salt to give 1.45g (23%) of product; mp. 253-257°C.

²⁰ ¹H-NMR (Me₂SO-d₆): δ 6.70-6.82 (m, 4, CH₂CH₂), 3.04 (br, s, 1, CHN), 7.35-7.55 (br, s, 2, NH₂), 8.13 (s, 1, CH), 9.49 (br s, 1, NHCH), 13.0-13.3 (br, s, 2, NH⁺₂).

25

Anal. Calcd. for C ₈ H ₁₀ N ₆ .HCl.I..H ₂ O:				
Calcd.:	C, 41.57;	H, 5.01;	N, 36.35;	Cl, 15.34.
Found:	C, 41.55;	H, 5.01;	N, 36.28;	Cl, 15.4.

2-Amino-6-(cyclopropylamino)-9-(2-deoxy-2-fluoro-β-ribofuranosyl)-9-purine

³⁰ ³⁵ 2-Amino-6-(cyclopropylamino)-9H-purine hydrochloride (0.3 g, 1.3 mmoles) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil (0.4 g, 1.6 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were dissolved in 20 ml of 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. Thymidine phosphorylase (2,000 I.U.) and purine nucleoside phosphorylase (5,540 I.U.) (T.A. Krenitsky et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,444) were added and the reaction incubated at 37°C. On day 5, 2,000 I.U. thymidine phosphorylase and 5,540 I.U. purine nucleoside phosphorylase were added. On day 21, the reaction was applied to a 2.5 x 8 cm column of anion exchange resin (Bio-Rad AGIX2-hydroxide form). After washing the column with water, the product was eluted with water/methanol (7/3). The product containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in water and lyophilized to obtain title compound that analyzed as a 0.6 hydrate.

⁴⁰ mp. 120°C (Partial melt at 80°).
UV λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1N HCl, 295.5 (14.3), 254 (12.7); pH 7, 282 (14.8) 262 (sh); 0.1N NaOH, 282 (15.2), 262 (sh).

45

Anal. Calcd. for C ₁₃ H ₁₇ FN ₆ O ₃ .0.6 H ₂ O:				
Calcd.:	C, 46.59;	H, 5.47;	N, 25.08;	F, 5.67.
Found:	C, 46.46;	H, 5.35;	N, 25.03;	F, 5.91.

⁵⁰ ⁵⁵ ¹H-NMR (200 mHz, Me₂SO-d₆): δ 7.93 (s, 1H, H-8); 7.38 (bd, 1H, 6-NH, J = 4.5 Hz); 6.04 (dd, 1H, H-1', JF,1'=16.4 Hz, J = 3.3 Hz); 5.88 (bs, 2H, 2-NH); 5.62 (d, 1H, 3'-OH, J = 5.9 Hz); 5.30 (apparent dt. 1H, H-2', JF,2 53.7 Hz, J 3.8 Hz); 5.23 (t, 1H, 5'-OH, J = 5.4 Hz); 4.38 (m, 1H, H-3'); 3.92 (m, 1H, H-4'); 3.69 (m, 1H, H_α-5'); 3.59 (m, 1H, H_β-5'); 3.10 (m, 1H, N-CH); 0.62 (m, 4H, CH₂CH₂).

Example 10

9-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)-6-methoxy-9H-purine

6-Methoxypurine (Sigma Chemical Company; 0.8 g, 5.3 mmoles) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil

(0.4 g, 1.6 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were suspended in 20 ml of 10 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. Thymidine phosphorylase (2,400 I.U.) and purine nucleoside phosphorylase (3,900 I.U.) (T.A. Krenitsky et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,344) were added and the suspension stirred at 37°C. On day 6 the reaction was diluted to 100 ml with 5mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. On day 16, 2,640 I.U. thymidine phosphorylase and 4,360 I.U. purine nucleoside phosphorylase were added. On day 45, the reaction was evaporated. The residue was suspended in warm methanol and the suspension was filtered. The filtrate was evaporated. The residue was dissolved in warm water and applied to a 2.5 x 7 cm column of anion exchange resin (Bio-Rad AG1X2-hydroxide form). After washing with water, the product was eluted with methanol/water (9/1). Product-containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in water and lyophilized to obtain title compound which analyzed as a 0.3 hydrate.

mp. 182°C UV λ max nm ($\epsilon \times 10^{-3}$): 0.1N HCl, 250 (8.72), 259 (sh); pH 7.248 (8.95), 259 (sh); 0.1N NaOH, 250 (9.14), 259 (sh).

Anal. Calcd. for $C_{11}H_{13}FN_4O_4 \cdot 0.3 H_2O$:				
Calcd.:	C, 45.61;	H, 4.73;	N, 19.34;	F, 6.56.
Found:	C, 45.72;	H, 4.66;	N, 19.47;	F, 6.72.

1H -NMR (300 MHz, Me_2SO-d_6): δ 8.63 and 8.58 (2s, 2H, H-8 and H-2); 6.34 (dd, 1H, H-1', JF,1' = 17.1 Hz, J = 2.4 Hz); 5.76 (d, 1H, 3'-OH, J = 6.1 Hz); 5.45 (ddd, 1H, H-2', JF,2' = 52.7 Hz, JL',2' = 2.4 Hz, J2',3' = 4.4 Hz); 5.17 (t, 1H, 5'-OH, J = 4.1 Hz); 4.50 (m, 1H, H-3'); 4.11 (s, 3H O-CH₃); 4.00 (m, 1H, H-4'); 3.75 (m, 1H, H_α-5'); 3.62 (m, 1H, H_β-5').

Example 11

2-Amino-9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-6-methoxy-9H-purine

2-Amino-6-methoxypurine (0.8 g, 4.8 mmoles) which may be prepared according to R.W. Balsiger and J.A. Montgomery (J. Org. Chem. 20:1573, 1960) and 1-(2-deoxy-2-fluoro- β -D-ribofuranosyl)uracil (0.4 g, 1.6 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were suspended in 20 of 10 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. Thymidine phosphorylase (2,400 I.U.) and purine nucleoside phosphorylase (3,900 I.U.) (T.A. Krenitsky, et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,344) were added and the suspension stirred at 37°C. On day 6, the reaction was diluted to 100 ml with 5mM potassium phosphate buffer, pH 7.0 which contained 0.04% (w/v) potassium azide. On day 10, the reaction was diluted to 200 ml with 5 mm potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. On day 16, 2,640 I.U. of thymidine phosphorylase and 4,360 I.U. of purine nucleoside phosphorylase were added. On day 24, the reaction was filtered and the filtrate was evaporated. The residue was suspended in methanol. The suspension was filtered and the filtrate evaporated. The residue was dissolved in water and applied to a 2.5 x 7 cm column of anion exchange resin (Bio-Rad AG1X2-hydroxide form). After washing the column with water, the product was eluted with methanol/water (9/1). After the solvent was removed under vacuum, the residue was dissolved in water and lyophilized to obtain title compound that analyzed as a 0.5 hydrate.

mp. 200-202°C UV λ max nm ($\epsilon \times 10^{-3}$): 0.1N HCl, 288 (7.85), 244.5 (6.43); pH 7, 279.5 (8.09), 248 (8.85); 0.1N NaOH, 280 (8.40). 248.5 (8.59).

Anal. Calcd. for $C_{11}H_{14}FN_5O_4 \cdot 0.5 H_2O$:				
Calcd.:	C, 42.86;	H, 4.90;	N, 22.78;	F, 6.16
Found:	C, 42.81;	H, 4.92;	N, 22.69;	F, 6.29

1H -NMR (300 MHz, Me_2SO-d_6): δ 8.12 (s, 1H, H-8); 6.57 (bs, 2H, 2-NH₂); 6.11 (dd, 1H, H-1', JF,1' = 16.6 Hz, J = 2.7 Hz); 5.69 (d, 1H, 3'-OH, J = 6.10 Hz); 5.31 (ddd, 1H, H-2', JF,2' = 53.0 Hz, J1',2' = 2.7 Hz, J2',3' = 4.4 Hz); 5.17 (t, 1H, 5'OH, J = 4.2 Hz); 4.40 (m, 1H, H-3'); 3.95 (m, 4H, H-4' and O-CH₃); 3.74 (m 1H, H_α-5'); 3.58 (m, 1H, H_β-5').

Example 12

6-Cyclopropylamino-9H-purine

A solution of 6-chloropurine (Aldrich Chemical Company, 4.23g, 27mmoles) and cyclopropylamine (Aldrich Chemical

Company, 12.5g, 22mmoles) in MeOH (100ml) was heated at 50°C for 48 hours. The solvent was removed, and the crude product was purified on a silica gel column eluting with CHCl₃: 5% MeOH to obtain 5.90g of a cream solid. This was recrystallized from MeOH to give two crops, 2.69g and 1.16g (81.4% total yield): mp. 237-240.

5 ¹H-NMR (Me₂SO-d₆): δ 0.6-071 (m, 4, CH₂CH₂), 3.03 (br s, 1, CHNH), 7.73 (d, 1, NH), 8.05 (s, 1, CH), 8.18 (s, 1, CH), 12.7 (br, 1, NH).

Anal. Calcd. C ₈ H ₉ N ₅ :	C, 54.85;	H, 5.18;	N, 39.97.
Found:	C, 54.69;	H, 5.22;	N, 39.87.

10 6-(Cyclopropylamino)-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine

15 6-(Cyclopropylamino)-9H-purine (0.2 g, 1.1 mmoles) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil (0.4 g, 1.6 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were suspended in 20 ml of 5mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. The pH of the suspension was adjusted to 7.0 with KOH. Thymidine phosphorylase (2,000 I.U.) and purine nucleoside phosphorylase (5,540 I.U.) (T.A. Krenitsky et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,344) were added and the suspension stirred at 37°C. On day 5, 0.2 g of 6-(cyclopropylamino)purine and 2,000 I.U. of thymidine phosphorylase and 5,540 I.U. of purine nucleoside phosphorylase were added. On day 9, 0.2 g of 6 (cyclopropylamino) purine was added and the pH of the reaction was adjusted to 7.0 with KOH. Thymidine phosphorylase (2,000 I.U.) and purine nucleoside phosphorylase (5,540 I.U.) were added. On day 20, the pH of the reaction was adjusted to 9.4 with NH₄OH and the reaction was applied to a 2.5 x 8.5 cm column of anion exchange resin (Bio-Rad AGIX2-hydroxide form). After washing with water, the product was eluted with water/methanol (7/3). Product-containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in water and lyophilized to obtain title compound that analyzed as a 0.4 hydrate.

25 mp. 207-208°C UV λmax nm (ε x 10-3): 0.1N HCl, 264 (19.1); pH 7, 263 (18.2); 0.1N NaOH, 268 (18.6).

Anal. Calcd. for C ₁₃ H ₁₆ FN ₅ O ₃ .0.4 H ₂ O:				
Calcd.:	C, 49.33;	H, 5.35;	N, 22.13;	F, 6.00.
Found:	C, 49.33;	H, 5.33;	N, 22.11;	F, 6.14.

30 ¹H-NMR (200 mHz, Me₂SO-d₆): δ 8.35 and 8.24 (2s, 2H, H-8 and H-2); 7.98 (bd, 1H, 6-NH, J = 4.10 Hz), 6.23 (dd, 1H, H-1', JF,1' = 16.8 Hz, J = 2.9 Hz); 5.68 (d, 1H, 3'-OH, J = 5.9 Hz); 5.42 (ddd, 1H, H-2', JF,2' = 53.0 Hz, J1',2' = 2.9 Hz, J2',3' = 4.6 Hz); 5.20 (t, 1H, 5-OH, J = 5.6 Hz); 4.46 (m, 1H, H-3'); 3.97 (m, 1H, H-4'); 3.73 (m, 1H, H_α-5'); 3.55 (m, 1H, H_β-5'); 3.04 (m, 1H, N-CH); 0.66 (m, 4H, CH₂CH₂).

35 Example 13

40 2-Amino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-6-ethoxy-9H-purine

45 2-Amino-6-ethoxypurine (0.8 g, 4.5 mmoles) which may be prepared according to R.W. Balsiger and J.A. Montgomery (J. Org. Chem. 25:1573, 1960) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil (0.5 g, 2.1 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were suspended in 50 ml of 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. The pH of the suspension was adjusted to 7.0 with KOH. Thymidine phosphorylase (4,000 I.U.) and purine nucleoside phosphorylase (14,000 I.U.) (T.A. Krenitsky et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,344) were added and the reaction stirred at 37°C. On day 4, 2,000 I.U. of thymidine phosphorylase and 6,980 I.U. of purine nucleoside phosphorylase were added and the reaction was diluted to 250 ml with 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. On day 50 14, the solvent was removed under vacuum. The residue was suspended in water and methanol added to precipitate the protein. After the suspension was filtered, the filtrate was evaporated. The residue was dissolved in hot water and applied to a 1.5 x 15 cm column of anion exchange resin (Bio-Rad AGIX2 hydroxide form). After washing the column with water, the product was eluted with methanol/water (1/1). Product-containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in water and lyophilized to obtain title compound that analyzed as 0.7 hydrate.

55 mp. 85°C (partial melt at 50°C) UV λmax nm (ε x 10-3): 0.1N HCl, 288 (8.78), 244.5 (7.19); pH 7, 280 (8.96), 247.5 (9.55); 0.1N NaOH, 230 (9.25), 249 (9.15).

5

Anal. Calcd. for C ₁₂ H ₁₆ FN ₅ O ₄ 0.7 H ₂ O:				
Calcd.:	C, 44.23;	H, 5.38;	N, 21.49;	F, 5.83.
Found:	C, 44.30;	H, 5.43;	N, 21.39;	F, 5.81.

¹H-NMR (200 mHz, Me₂SO-d6): δ 8.09 (s, 1H, H-8); 6.49 (bs, 2H, 2-NH2); 6.08 (dd, 1H, H-1', JF,1' = 16.4 Hz, J = 2.7 Hz); 5.67 (d, 1H, 3'-OH, J = 6.1 Hz); 5.42 (m, 0.5H, 0.5 (H-2')); 5.15 (m, 1.5H, 0.5 (H-2') and 5'-OH); 4.44 (q, 2H, 6-OCH₂, J = 7.0 Hz); 4.40 (m, 1H, H-3'); 3.92 (m, 1H, H-4'); 3.73 (m, 1H, H_α-5'); 3.56 (m, 1H, H_β-5'); 1.34 (t, 3H, -CH₃, J = 7.0 Hz).

10

Example 14

2-Amino-6-chloro-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine

15 Reaction 1: 2-Amino-6-chloropurine (Sigma Chemical Company, 0.8 g, 4.7 mmoles) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil (0.4 g, 1.6 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were suspended in 50 ml of 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. The pH of the suspension was adjusted to 7.0 with KOH. Thymidine phosphorylase (3,850 I.U.) and purine nucleoside phosphorylase (6,500 I.U.) (T.A. Krenitsky et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,444) were added and the suspension stirred at 37°C. On day 22, the reaction was diluted to 100 ml with 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide, and 1,270 I.U. thymidine phosphorylase and 2,180 I.U. purine nucleoside phosphorylase were added. On day 32, the reaction was diluted to 200 ml with 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide, and 2,540 I.U. of thymidine phosphorylase and 4,360 I.U. of purine nucleoside phosphorylase were added. On day 61, the reaction was filtered. The filtrate was evaporated and the residue stored at 4°.

20 Reaction 2: 2-Amino-6-chloropurine (Sigma Chemical Company, 0.8 g, 4.7 mmoles) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil (0.4 g, 1.6 mmoles) were suspended in 25 ml of 10 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. Thymidine phosphorylase (4,000 I.U.) and purine nucleoside phosphorylase (6,500 I.U.) were added and the suspension stirred at 37°C. On day 10, the reaction was diluted to 100 ml with 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. On day 20, the reaction was diluted to 200 ml with 5 mM potassium phosphate buffer, pH 7, which contained 0.04% (w/v) potassium azide, and 2,640 I.U. thymidine phosphorylase and 4,360 I.U. purine nucleoside phosphorylase were added. On day 53, the reaction was filtered. The filtrate was evaporated and the residue stored at 4°. The residues from Reactions 1 and 2 were suspended in water and combined. The suspension was warmed and then filtered. The product which was contained in the filtrate was purified by chromatography on a 7.5 x 90 cm column of Biogel P-2 (Bio-Rad) with n-propanol/water (3/7) as the solvent, followed by chromatography on a 5 x 90 cm column of Sephadex G-10 (Pharmacia LKB) with n-propanol/water (3/7) as the solvent. Fractions containing only product were pooled and the solvent removed under vacuum. The residue was suspended in water and lyophilized to obtain the title compound (batch 1). Fractions containing product plus impurities which were eluted off the Sephadex G-10 column were combined and the solvent removed under vacuum. The residue was dissolved in water/MeCN (49/1), and the product was further purified by reversed phase chromatography on C18 silica (Hi-Chrom Prep-40-ODS, Regis Chemical Co.) with water/MeCN (49/1) as the solvent. Product containing fractions were combined and filtered through an 0.2 μm-pore nylon filter to remove residual silica. After the solvent was removed under vacuum, the residue was dissolved in water and filtered through a 0.22 m-pore membrane filter (Millipore GS). Lyophilization of the filtrate yielded the title compound (batch 2) that analyzed as a 0.5 hydrate.

45

Data for batch 1:

mp. 212°C (partial melt at 205°C) UV λ_{max} nm (ε X10-3): 0.1N HCl, 309 (6.00), 247 (5.60); pH 7, 307.5 (6.30), 247 (5.80); 0.1N NaOH, 307 (6.40), 247 (5.30).

50

Anal. Calcd. for C ₁₀ H ₁₁ Cl FN ₅ O ₃ :					
Calcd.:	C, 39.55;	H, 3.65;	N, 23.06;	Cl, 11.67;	F, 6.26.
Found:	C, 39.69;	H, 3.82;	N, 22.84;	Cl, 11.64;	F, 6.14.

55

¹H-NMR (300 mHz, Me₂SO-d6): δ 8.37 (s, 1H, H-8); 7.07 (bs, 2H, 2-NH2); 6.12 (dd, 1H, H-1', JF,1' = 16.6Hz, J = 2.0 Hz); 5.72 (d, 1H, 3'-OH, J = 6.4 Hz); 5.34 (ddd, 1H, H-2', JF,2' = 52.9 Hz, J1',2' = 2.0 Hz, J2',3' = 4.2 Hz); 5.19 (t, 1H, 5'-OH, J = 5.3 Hz); 4.42 (m, 1H, H-3'); 3.96 (m, 1H, H-4'); 3.76 (m, 1H, H_α-5'); 3.61 (m, 1H, H_β-5').

Data for batch 2:

mp. 215°C UV λ_{max} nm: 0.1N HCl, 309.5, 246.5; pH 7, 307.5, 247; 0.1N NaOH, 307.5, 247.

5	Anal. Calcd. for $C_{10}H_{11}ClFN_5O_3 \cdot 0.5 H_2O$:					
	Calcd.:	C, 38.41;	H, 3.87;	N, 22.40;	Cl, 11.34;	F, 6.08.
	Found:	C, 38.73;	H, 3.79;	N, 22.14;	Cl, 11.16;	F, 6.10.

10 $^1\text{H-NMR}$ (80 mHz, $\text{Me}_2\text{SO-d}6$): 8.36 (s, 1H, H-8); 7.03 (bs, 2H, 2-NH2); 6.13 (dd, 1H, H-1', JF,1' = 16.8 Hz, J = 3.2 Hz); 5.68 (m, 1.5H, 0.5 (H-2') and 3'-OH); 5.15 (m, 1.5H, 0.5 (H-2') and 5'-OH); 4.35 (m, 1H, H-3'); 3.90 (m, 1H, H-4'); 3.72 (m, 2H, H_{α} -5' and H_{β} -5').

Example 15

15 2-Amino-9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-6-methylamino-9H-purine

2-Amino-6-Amino-6-methylaminopurine (J.A. Montgomery and L.B. Holum, J.A.C.S. 80:404, 1958; 0.51 g; 3.1 mmoles) and 1-(2-deoxy-2-fluoro- β -D-ribofuranosyl)uracil (0.52 g, 2.1 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were suspended in 50 ml of 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. Thymidine phosphorylase (4,000 I.U.) and purine nucleoside phosphorylase (14,000 I.U.) (T.A. Krenitsky et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,344) were added and the suspension stirred at 37°C. On day 6, the reaction was diluted to 250 ml with 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide and 0.49 g of 2-amino-6-methylaminopurine and 4,000 I.U. thymidine phosphorylase and 14,000 I.U. purine nucleoside phosphorylase were added. On day 14, the reaction was evaporated. The residue was suspended in methanol/water and the suspension filtered. The filtrate was evaporated. The residue was dissolved in water and applied to a 2.5 x 13 cm column of anion exchange resin (Bio-Rad AG1X2-hydroxide form). After washing the column with water, the product was eluted with methanol/water (1/1). After the solvent was removed under vacuum, the residue was dissolved in water and filtered through a 0.22 μm -pore membrane filter. Lyophilization of the filtrate yielded title compound that analyzed as a 0.5 hydrate.

30 mp. 172°C UV λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1N HCl, 292.5 (11.5), 255 (12.1); pH 7, 279.5 (13.4), 263 (sh); 0.1N NaOH, 280 (13.7), 263 (sh).

35 **Anal. Calcd. for $C_{11}H_{15}FN_6O_3 \cdot 0.5 H_2O$:**

Calcd.: C, 43.00; H, 5.25; N, 27.35; F, 6.18.

Found: C, 42.99; H, 5.28; N, 27.33; F, 6.16.

40 $^1\text{H-NMR}$ (200 mHz, $\text{Me}_2\text{SO-d}6$): δ 7.92 (s, 1H, H-8); 7.28 (bs, 1H, 6-NH); 6.13 (dd, 1H, H-1', JF,1' = 16.3 Hz, J = 3.3 Hz); 5.91 (bs, 2H, 2-NH2); 5.64 (d, 1H, 3'-OH, J = 5.8 Hz); 5.29 (ddd, 1H, H-2', JF,2' = 53.0 Hz, J1',2' = 3.3 Hz, J2',3' = 4.4 Hz); 5.27 (t, 1H, 5'-OH, J = 5.5 Hz); 4.37 (m, 1H, H-3'); 3.92 (m, 1H, H-4'); 3.72 (m, 1H, H_{α} -5'); 3.60 (m, 1H, H_{β} -5'; 2.86 (bs, 3H, N-CH3).

Example 16

45 9-(2-Deoxy-2-fluoro- β -D-ribofuranosyl)hygoxanthine

50 6-Amino-9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-9H-purine (0.29 g, 1.0 mmole), prepared as in Example 8, was dissolved in 100 ml of water. Calf intestinal adenosine deaminase (4 I.U., Boehringer Mannheim) was added and the solution incubated at 37°C for 24 hrs. The solvent was removed under vacuum. The residue was dissolved in water/n-propanol (7/3) and chromatographed on a 5 x 90 cm column of Sephadex G.10 (Pharmacia LKB) with water/n-propanol (7/3) as the solvent. Product-containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in water and lyophilized to obtain title compound that analyzed as a hydrate.

55 mp. 175°C (partial melt at 125°) UV λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1N HCl, 249 (11.8); pH 7, 248.5 (12.0); 0.1N NaOH, 253 (13.0).

5

Anal. Calcd. for C ₁₀ H ₁₁ FN ₄ O ₄ H ₂ O:				
Calcd.:	C, 41.67;	H, 4.55;	N, 19.44;	F, 6.59.
Found:	C, 41.72;	H, 4.58;	N, 19.46;	F, 6.37.

¹⁰ ¹H-NMR (300 mHz, Me₂SO-d6): δ 8.33 and 8.09 (2s, 2H, H-8 and H-2); 6.21 (dd, 1H, H-1', JF,1' = 16.8 Hz, J = 2.4 Hz); 5.75 (bs, 1H, 3'-OH), 5.36 (ddd, 1H, H-2', JF,2' = 52.7 Hz, J1',2' = 2.4 Hz, J1',3' - 4.4 Hz); 5.20 (bs, 1H, 5'-OH); 4.43 (apparent d m, 1H, H-3', JF,3' - 18.9 Hz); 3.97 (m, 1H, H-4'); 3.75 (m, 1H, H_α-5'); 3.59 (m, 1H, H_β-5')

¹⁵ Example 17

2-Amino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-6-propoxy-9H-purine

²⁰ 2-Amino-6-propoxypurine (0.3 g, 1.6 mmoles) which may be prepared according to R.W. Balsiger and J.A. Montgomery (J. Org. Chem. 25:1573, 1960) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil (0.52 g, 2.1 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were suspended in 50 ml of 2 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. The pH of the suspension was adjusted to 7.0 with KOH. Thymidine phosphorylase (4,000 I.U.) and purine nucleoside phosphorylase (14,000 I.U.) (T.A. Krenitsky et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,344) were added and the reaction stirred at 37°C. On day 6, the reaction was diluted to 250 ml with 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide, and 4,000 I.U. of thymidine phosphorylase and 14,000 I.U. of purine nucleoside phosphorylase were added. On day 14, the solvent was removed under vacuum. The residue was suspended in methanol/water and the suspension was filtered. The filtrate was evaporated. The residue was dissolved in hot water and applied to a 2.5 x 13 cm column of anion exchange resin (Bio-Rad AG1X2 hydroxide form). After washing the column with water, the product was eluted with methanol/water (1/1). Product-containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in water and lyophilized to obtain title compound that analyzed as 0.9 hydrate.

²⁵ mp. 93°C (partial melt at 65°C) UV λ_{max} nm (ε x 10-3): 0.1N HCl, 289 (9.05), 245 (7.37); pH 7, 280 (9.28), 248 (9.82); 0.1N NaOH, 280.5 (9.67), 249.5 (9.55).

Anal. Calcd. for C₁₃H₁₈FN₅O₄ 0.9 H₂O:
Calcd.: C, 45.69; H, 5.78; N, 20.49; F, 5.56.

³⁵ **Found: C, 45.75; H, 5.61; N, 20.51; F, 5.60.**

⁴⁰ ¹H-NMR (200 mHz, Me₂SO-d6): δ 8.09 (s, 1H, H-8); 6.49 (bs, 2H, 2-NH2); 6.08 (dd, 1H, H-1, JF,1' = 16.4 Hz, J = 2.7 Hz); 5.65 (d, 1H, 3'-OH, J = 6.0 Hz); 5.42 (apparent t, 0.5H, 0.5 (H-2'), J = 3.5 Hz); 5.14 (m, 1.5H, 0.5 (H-2') and 5'-OH); 4.36 (m, 3H, H-3'; and 6-OCH₂); 3.92 (m, 1H, H-4'); 3.69 (m, 1H, H_α-5'); 3.60 (m, 1H, H_β-5'); 1.75 (apparent sextet, 2H, CH₂, J = 7.1 Hz); 0.95 (t, 3H, CH₃, J = 7.4 Hz).

Example 18

2-Amino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-6-iodo-9H-purine

⁴⁵ 2-Amino-6-iodopurine (0.8 g, 3.1 mmoles) which may be prepared according to R.T. Koda et al. (J. Pharm. Sci. 57: 2056, 1968) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil (0.39 g, 1.6 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were suspended in 20 ml of 10 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. The pH of the suspension was adjusted to 7.2 with KOH. Thymidine phosphorylase (2,000 I.U.) and purine nucleoside phosphorylase (3,250 I.U.) (T.A. Krenitsky et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,344) were added and the suspension stirred at 37°C. On day 12, the pH of the reaction was adjusted to 6.8 with acetic acid, and 2,000 I.U. of thymidine phosphorylase and 3,250 I.U. of purine nucleoside phosphorylase were added. On day 26, the reaction was diluted to 500 ml with 5 mM potassium phosphate buffer, pH 7, which contained 0.04% (w/v) potassium azide, and 2,000 I.U. thymidine phosphorylase and 3,250 I.U. purine nucleoside phosphorylase were added. On day 40, the reaction was filtered. The filtrate was evaporated. The residue was suspended in water/n-propanol (7/3) and filtered. The filtrate was evaporated. The residue was suspended in methanol and then filtered. The product which was contained in the filtrate was purified by chromatography on a 5 x 90 cm column of Biogel P-2 (Bio-Rad) with water/n-propanol (7/3) as the solvent, followed by chromatography on a 5 x

90 cm column of Sephadex G-10 (Pharmacia LKB) with water/n-propanol (7/3) as the solvent. After the solvent was removed under vacuum, the residue was suspended in water and lyophilized to obtain title compound that analyzed as a 0.6 hydrate.

mp. 135°C (partial melt at 108-110°C) UV λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1N HCl, 318 (7.94); pH 7, 315 (8.30); 0.1N NaOH, 315 (8.36).

Anal. Calcd. for $C_{10}H_{11}FN_5O_3$ 0.6 H_2O :					
Calcd.:	C, 29.59;	H, 3.03;	N, 17.25;	F, 4.68;	I, 31.26.
Found:	C, 29.69;	H, 3.06;	N, 17.22;	F, 4.44;	I, 31.47.

1H -NMR (300 mHz, Me_2SO-d_6): δ 8.33 (s, 1H, H-8); 6.96 (bs, 2H, 2-NH2); 6.09 (dd, 1H, H-1', $JF,1' = 16.7$ Hz, $J = 2.4$ Hz); 5.69 (d, 1H, 3'-OH, $J = 6.3$ Hz); 5.33 (ddd, 1H, H-2', $JF,2' = 52.8$ Hz, $J1',2' = 2.4$ Hz, $J2',3' = 4.2$ Hz); 5.16 (t, 1H, 5'-OH, $J = 5.3$ Hz); 4.42 (m, 1H, H-3'); 3.95 (m, 1H, H-4'); 3.77 (m, 1H, $H_{\alpha}, .5'$); 3.60 (m, 1H, $H_{\beta}, -5'$).

Example 19

9-(2-Deoxy-2-fluoro- β -D-ribofuranosyl)-6-methylamino-9H-purine

6-Methylaminopurine (Sigma Chemical Company, 0.8 g, 5.4 mmoles) and 1-(2-deoxy-2-fluoro- β -D-ribofuranosyl)uracil (0.39 g, 1.6 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were suspended in 20 ml of 10 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. Thymidine phosphorylase (2,400 I.U.) and purine nucleoside phosphorylase (3,900 I.U.) (T.A. Krenitsky et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,344) were added and the suspension stirred at 37°C. On day 6, the reaction was diluted to 100 ml with 5mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. On day 17, the reaction was filtered. The filtrate was evaporated. The residue was suspended in methanol and then filtered. The filtrate was evaporated. The residue was dissolved in water and applied to a 2.5 x 7 cm column of anion exchange resin (Bio-Rad AGIX2-hydroxide form). The product was eluted with water. Product-containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in water and filtered through a 0.2 μ m-pore membrane filter. Lyophilization of the filtrate yielded title compound.

mp. 140°C (shrinks at 65°, partial melt at 110°) UV λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1N HCl, 261.5 (16.2); pH 7, 265.5 (15.0); 0.1N NaOH, 266 (15.3).

Anal. Calcd. for $C_{11}H_{14}FN_5O_3$:				
Calcd.:	C, 46.64;	H, 4.98;	N, 24.72;	F, 6.71.
Found:	C, 46.48;	H, 5.07;	N, 24.63;	F, 6.93.

1H -NMR (300 mHz, Me_2SO-d_6): δ 8.37 and 8.25 (2s, 2H, H-8 and H-2); 7.86 (bs, 1H, 6-NH); 6.24 (dd, 1H, H-1', $JF,1' = 16.8$ Hz, $J = 3.2$ Hz); 5.74 (d, 1H, 3'-OH, $J = 6.1$ Hz); 5.44 (ddd, 1H, H-2', $JF,2' = 53.0$ Hz, $J1',2' = 3.2$ Hz, $J2',3' = 4.4$ Hz); 5.28 (t, 1H, 5'-OH, $J = 5.6$ Hz); 4.49 (m, 1H, H-3'); 3.99 (m, 1H, H-4'); 3.75 (m, 1H, $H_{\alpha}, -5'$); 3.58 (m, 1H, $H_{\beta}, -5'$); 2.95 (bs, 3H, N-CH3).

Example 20

9-(2-Deoxy-2-fluoro- β -D-ribofuranosyl)-6-ethoxy-9H-purine

6-Ethoxypurine (Sigma Chemical Company; 0.2 g, 1.2 mmoles) and 1-(2-deoxy-2-fluoro- β -D-ribofuranosyl)uracil (0.4 g, 1.6 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were suspended in 20 ml of 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. The pH of the suspension was adjusted to 7.0 with KOH and thymidine phosphorylase (2,000 I.U.) and purine nucleoside phosphorylase (5,540 I.U.) (T.A. Krenitsky et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,344) were added and the suspension stirred at 37°C. On day 5, an additional 2,000 I.U. thymidine phosphorylase and 2,770 I.U. of purine nucleoside phosphorylase were added. On day 9, 0.2 g 6-ethoxypurine was added. The pH of the reaction was adjusted to 7.0 with KOH and 2,000 I.U. thymidine phosphorylase and 5,540 I.U. purine nucleoside phosphorylase were added. On day 28, the suspension was filtered. The filtrate was applied to a 2.5 x 8.5 cm column of anion exchange resin (Bio-Rad AGIX2-hydroxide form). After washing with water, the product was eluted with methanol/water (3/7). Product containing fractions were combined and the solvent removed under vacuum. The product contained in the residue was

further purified by chromatography on a 2.5 x 90 cm column of Biogel P-2 (Bio-Rad) with water/methanol (8/2) as the solvent followed by chromatography on a 2.5 x 50 cm silica gel column with acetonitrile/water (49/1) as the solvent. Product containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in water and lyophilized to obtain title compound which analyzed as a 0.5 hydrate.

5 mp. 85-87°C (partial melt at 60°) UV λ max nm ($\epsilon \times 10^{-3}$): 0.1N HCl, 249 (11.4); pH 7, 248 (11.7): 0.1N NaOH, 249 (12.0).

Anal. Calcd. for $C_{12}H_{15}FN_4O_4$ 0.5 H_2O :					
Calcd.:	C, 46.91;	H, 5.25;	N, 18.23;	F, 6.18.	
Found:	C, 46.80;	H, 5.23;	N, 18.21;	F, 6.65.	

10 1H -NMR (200 mHz, Me_2SO-d_6): δ 8.59 and 8.53 (2s, 2H, H-8 and H-2); 6.31 (dd, 1H, H-1', JF,1' = 7.0 Hz, J = 2.5 Hz); 5.70 (d, 1H, 3'-OH, J = 6.3 Hz); 5.43 (ddd, 1H, H-2', JF,1' = 52.9 Hz, J1',2' = 2.5 Hz, J2',3' = 4.4 Hz); 5.12 (t, 1H, 5'-OH, J = 5.4 Hz); 4.59 (q, 2H, 6-OCH₂, J = 7.0 Hz); 4.51 (m, 1H, H-3'); 3.98 (m, 1H, H-4'); 3.73 (m, 1H, H_{α} -4'); 3.61 (m, 1H, H_{β} -5'); 1.40 (t, 3H, -CH₃, J = 7.0 Hz).

Example 21

2-Amino-9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-6-methylthio-9H-purine

20 2-Amino-6-methylthiopurine (Sigma Chemical Company; 0.6 g, 3.3 mmoles) and 1-(2-deoxy-2-fluoro- β -D-ribofuranosyl)uracil (0.3 g, 1.2 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:558, 1964) were suspended in 200 ml of 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. 25 Thymidine phosphorylase (4,000 I.U.) and purine nucleoside phosphorylase (6,500 I.U.) (T.A. Krenitsky et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,444) were added and the suspension stirred at 37°C. On day 14, the reaction was diluted to 400 ml with 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide and 8,000 I.U. thymidine phosphorylase and 6,500 I.U. purine nucleoside phosphorylase were added. On day 25, the pH of the reaction was adjusted to 6.9 with KOH. On day 39, the reaction was filtered. The filtrate was applied to a 2.5 x 13 cm column of anion exchange resin (Bio-Rad AG1X2-hydroxide form) and the product was eluted with methanol/water (3/7). After the solvent was removed under vacuum, the residue was suspended in water and lyophilized to obtain title compound that analyzed as a 0.5 hydrate.

30 mp. 85°-87°C UV λ max nm ($\epsilon \times 10^{-3}$): 0.1N HCl, 327 (11.6), 250 (11.2), 263 (sh); pH 7, 311 (12.8), 257 (sh), 246 (15.1); 0.1N NaOH, 311 (13.3), 257 (sh), 246 (14.8).

Anal. Calcd. for $C_{11}H_{14}FN_5O_3S$ 0.5 H_2O :					
Calcd.:	C, 40.74;	H, 4.66;	N, 21.59;	F, 5.80;	S, 9.89.
Found:	C, 40.79;	H, 4.66;	N, 21.55;	F, 5.89;	S, 9.84.

40 1H -NMR (80 mHz, Me_2SO-d_6): δ 8.17 (s, 1H, H-8); 6.57 (bs, 2H, 2-NH₂); 6.14 (dd, 1H, H-1', JF,1' = 16.4 Hz, J = 3.2 Hz); 5.64 (m 1.5H, 0.5 (H-2') and 3'-OH); 5.11 (m, 1.5H, 0.5 (H-2') and 5'-OH); 4.40 (m 1H, H-3'); 3.95 (m, 1H, H-4'); 3.65 (m, 1H, H_{α} -5' and H_{β} -5'); 2.58 (s, 3H, 6-SCH₃).

Example 22

9-(2-Deoxy-2-fluoro- β -D-ribofuranosyl)-6-iodo-9H-purine

45 6-Iodopurine (Sigma Chemical Company; 0.7 g, 2.7 mmoles) and 1-(2-deoxy-2-fluoro- β -D-ribofuranosyl)uracil (0.4 g, 1.7 mmoles) which may be prepared according to J.F. Codington et al. (J. Org. Chem. 29:556, 1964) were suspended in 20 ml of 10 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. Thymidine phosphorylase (2,640 I.U.) and purine nucleoside phosphorylase (4,360 I.U.) (T.A. Krenitsky et al., Biochemistry 20:3615, 1981 and U.S. Patent 4,381,344) were added and the suspension stirred at 37°C. On day 21, the reaction was diluted to 50 ml with 5 mM potassium phosphate buffer, pH 7, which contained 0.04% (w/v) potassium azide and 4000 I.U. of thymidine phosphorylase and 6,500 I.U. of purine nucleoside phosphorylase were added. On day 43, the reaction was diluted to 250 ml with water and 2,000 I.U. thymidine phosphorylase and 3,250 I.U. purine nucleoside phosphorylase were added. On day 57, the reaction was filtered. The filtrate was evaporated. The residue was suspended in warm methanol and filtered. The filtrate was evaporated. The residue was dissolved in water/n-propanol (7/3) and applied to

a 7.5 x 90 cm column of Biogel P-2 (Bio-Rad) with water/n-propanol (7/3) as the solvent. Product containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in hot methanol and water added until a precipitate formed. The methanol was removed under vacuum. After standing overnight the suspension was filtered. The filter cake contained the bulk of the product. The filtrate was evaporated and the above procedure repeated to precipitate the remaining product. The filter cakes were combined and suspended in water. Lyophilization yielded the title compound.

5 mp. 198-200°C (dec.)
 UV λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1N HCl, 275 (11.1), 257 (sh); pH 7, 275 (11.1), 258 (sh); 0.1N NaOH, 276 (9.98), 257 (sh),
 10 303 (sh).

Anal. Calcd. for $C_{10}H_{10}FN_4O_3$					
Calcd.:	C, 31.60;	H, 2.65;	N, 14.74;	F, 5.00;	I, 33.39.
Found:	C, 31.70;	H, 2.70;	N, 14.69;	F, 4.96;	I, 33.48.

15 $^1\text{H-NMR}$ (80 mHz, $\text{Me}_2\text{SO-d}6$): δ 8.87 and 8.66 (2s, 2H, H-8 and H-2); 6.35 (dd, 1H, H-1', JF,1' = 17.0 Hz, J = 2.0 Hz); 5.76 (m, 1.5H, 0.5 (H-2') and 3'-OH); 5.14 (m, 1.5H, 0.5 (H-2') and 5'-OH); 4.51 (m, 1H, H-3'); 3.99 (m, 1H, H-4'); 3.70 (m, 2H, $H_{\alpha}\text{-5}'$ and $H_{\beta}\text{-5}'$).

20 Example 23

Preparation of 9-(2-Deoxy-2-fluoro-5-O-L-valinyl- β -D-ribofuranosyl)-adenine and its bis-hydrochloride salt

25 To a solution of 9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)adenine (500 mg, 1.86 mmol), FMOC-L-valine (820 mg 1.3 eq) and DMAP (10 mg) in dry DMF (14 ml) at 0°C was added a solution of DCC (Dicyclohexylcarbodiimide)(520 mg, 1.35 eq) in CH_2Cl_2 (2 ml) with stirring. The mixture was allowed to attain room temperature, stirred for 90 minutes and then evaporated in vacuo. After co-evaporation with ethanol the residue was taken up in $\text{CHCl}_3/\text{MeOH}$ (9:1) and filtered. The filtrate was then evaporated and chromatographed over SiO_2 using the flash technique, eluting with a gradient of EtOH in CHCl_3 (5%-10%).

30 The first eluted fraction was the 3',5'-bis ester (230 mg) containing some dicyclohexylurea. The second fraction was the 3'-mono ester and the third fraction was the 5'-mono ester (190 mg).

35 The 5'-FMOC protected valinate ester was then treated with a solution of piperidine (1 ml) in DMF (4 ml) at room temperature for 5 minutes, evaporated in vacuo, dissolved in water (25 ml) and washed with CHCl_3 (30 ml). The water was evaporated to produce a colourless gum, which was taken up in aqueous acetic acid, evaporated, and co-evaporated with ethanol and ether to provide the 5'-O-valinate ester as a hygroscopic amorphous solid (100 mg).

Anal. $C_{15}H_{21}FN_6O_4 \cdot 1.5 CH_3CO_2H \cdot 1.5 H_2O$			
requires:	C 44.53;	H 6.23;	N 17.32
Found:	C 44.38;	H 6.32;	N 16.94

40 mp. Decomposes above 150°C.

45 The bis-hydrochloride salt was prepared by dissolving 5'-O-valinate ester in isopropanol followed by the addition of isopropanol previously saturated with hydrogen chloride gas, and evaporation of the solvent. The white solid was then taken up in MeOH and precipitated with ether at 0°C. Filtration provided the bis-hydrochloride salt.

Anal. $C_{15}H_{21}FN_6O_4 \cdot 2HCl \cdot 0.5 H_2O$			
requires:	C 40.00;	H 5.37;	N 18.67
Found:	C 40.26;	H 5.19;	N 18.74

50 mp. 210-213° (dec.)

55 Example 24

2-Amino-9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-9H-purine bis hydrochloride

To a solution of 2-amino-9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-9H-purine (300mg 1.11 mmol) in MeOH (20ml) was

added isopropanol (2.5 ml) previously saturated with hydrochloride (HCl) gas. Acetone (15 ml) was added and the solution evaporated to almost dryness under vacuum at room temperature. Trituration with ethyl acetate (15 ml) produced a white solid which was filtered and washed with ether.

mp. 160-163° (dec)

5

Anal. C ₁₀ H ₁₂ F N ₅ O ₃ 2 HCl				
requires:	C 35.01;	H, 4.12;	N, 20.42;	F, 5.55
Found:	C 34.79;	H, 4.23;	N, 20.32;	F, 5.66

10

Example 25

2,6-Diamino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine bis hydrochloride

15 To a solution of 2,6-diamino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine (300mg, 1.06 mmol) in MeOH (30 ml) was added isopropanol (2 ml) previously saturated with HCl gas. The solution was evaporated to a small volume (5 ml) at room temperature, and EtOH (15 ml) added to precipitate the bis hydrochloride as a white solid.

mp. 165-168°C (dec)

20

Anal. C ₁₀ H ₁₃ FN ₆ O ₃ . 2 HCl				
requires:	C, 33.62;	H, 4.23;	N, 23.53;	F, 5.32
Found:	C, 33.54;	H, 4.24;	N, 22.98;	F, 5.33

25

Example 26

9-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)adenine hydrochloride

30 To a solution of 9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)adenine (500 mg, 1.86 mmol) in MeOH (40 ml) and water (10 ml) was added a solution of isopropanol (15 ml) previously saturated with HCl gas. The solution was evaporated under vacuum at room temperature, and triturated with EtOH (25 ml) to afford the title compound as a white solid.

mp. 205-210° (dec)

35

Anal. C ₁₀ H ₁₂ FN ₅ O ₃ HCl				
requires:	C, 39.29;	H, 4.29;	N 22.91;	CL, 11.60
Found:	C, 39.36;	H, 4.34;	N 22.89;	CL, 11.52

Example 27

40

9-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)adenine-5'-monophosphate

45 9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)adenine (of Example 8, 0.47 g, 1.7 mmol) was dissolved in 7 ml of 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone. After cooling this solution to -8°C in an ice/methanol bath, 0.64 ml phosphorus oxychloride (7 mmol) was added with vigorous stirring. After three minutes the reaction was stopped by adding 10 ml cold water. This reaction mixture was kept on ice for 15 min, then neutralized to pH 8 with ammonium hydroxide.

50 Separation of the reaction products was performed using anion exchange chromatography on DEAE Sephadex A-25. The reaction mixture was diluted to 600 ml with water and applied to a chromatography column containing about 80 ml of DEAE Sephadex A-25 which had been previously equilibrated in 50 mM ammonium bicarbonate. The column was washed with 2.5 L of 50 mM ammonium bicarbonate to remove inorganic phosphate. The nucleotides were eluted with a 2 L linear gradient of 50 - 500 mM ammonium bicarbonate. 9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)adenine-5'-monophosphate eluted first followed by 9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)adenine-3',5'-bisphosphate. Fractions containing each nucleotide were pooled and dried in vacuo to remove water and ammonium bicarbonate.

55 9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)adenine-5'-monophosphate was obtained as the ammonium salt from the above scheme but the sodium salt was the pharmacologically desired salt in this case. The ammonium salt was exchanged for sodium using Dowex 50 ion exchange resin. 9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)adenine-5'-monophosphate (1.3 mmol) in 10 ml water was applied to a column containing about 10 ml of BioRad AG50W-X8 (sodium form) which had been previously equilibrated with water. The nucleotide was eluted with water. Fractions containing 9-(2-de-

oxy-2-fluoro- β -D-ribofuranosyl)adenine-5'-monophosphate combined and lyophilized to give 0.49 g (1.2 mmol, 72 % yield). $^1\text{H-NMR}$ d (d_6 -DMSO) δ 3.0 (1H, s, H2), 7.7 (1H, s, H8), 5.7 and 5.9 (1H, dd, H1', split by H2',F), 4.8 and 5.0 (1H dd, H2', split by H1',F), 4.1 (1H, m, H3'), 3.9 (1H, m, H4'), 3.6 (2H, m, H5') $^1\text{H-NMR}$ d (D_2O) 8.5 (1H, s, H2), 8.2 (1H, s, H8), 6.3 and 6.5 (1H, d, H1', split by H2',F), 5.3 and 5.6 (1H, d, H2', split by H1',F), 4.7 (1H, m, H3'), 4.4 (1H, m, H4'), 4.1 (2H, m, H5') ^{31}P NMR δ (D_2O) 1.46 (s) UV spectra: in 0.1 M HCl λ_{max} at 256 nm; in 400 mM ammonium phosphate, pH 5.5 λ_{max} at 259 nm; in 50 mM potassium phosphate, pH 7.0 max at 259 nm; in 0.1 M NaOH λ_{max} at 259 nm.

Mass spectrum gave two main peaks at molecular ion fragment weights of 270 and 136, corresponding to 9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-adenine, respectively.

Total cleavage to the nucleoside was observed after incubation with 5'-nucleotidase (Sigma).

Base/phosphate ratio= 1.00/1.03. The concentration of total phosphate was determined by the method of Ames, 3.N. in Methods in Enzymology Vol. 8 pp. 115-118, 1960. The concentration of nucleobase was determined using the UV extinction coefficient of the nucleoside.

15 Example 28

9-(2-Deoxy-2-fluoro- β -D-ribofuranosyl)adenine-5'-monophosphate (FMAP)

20 9-(2-Deoxy-2-fluoro- β -D-ribofuranosyl)adenine (of Example 8, 1.2 mg, 4.3 μmol), 22 μmol p-nitrophenylphosphate (1M stock adjusted to pH 5.4 with acetic acid) and 0.05 ml of nucleoside phosphotransferase from Serratia marcescens [A. Fyfe, et al., *J.Biol.Chem.* 253, 8721-8727 (1978) and US Patent 4,136,175 Jan.23, 1979] were combined with water to give a final volume of 0.22 ml. Incubation was done at 37°C overnight. The entire reaction was spotted onto a preparative cellulose thin layer chromatography plate then developed in n-propanol/15 M $\text{NH}_4\text{OH}/\text{H}_2\text{O}$: 6/3/1. After drying the plate, the area containing nucleotide was scraped off and the nucleotide was eluted from the cellulose with water.

25 9-(2-Deoxy-2-fluoro- β -D-ribofuranosyl)adenine -5'-monophosphate was obtained in 25% yield, 1.1 μmol .

UV spectra: in water, λ_{max} at 257 nm.

This compound was completely cleaved by alkaline phosphatase and 5'-nucleotidase to give the nucleoside 9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)adenine.

Base/phosphate ratio- 1/8, indicating contamination by inorganic phosphate.

30 Example 29

9-(2-Deoxy-2-fluoro- β -D-ribofuranosyl)adenine-3' 5'-bisphosphate

35 The 9-(2-Deoxy-2-fluoro- β -D-ribofuranosyl)adenine-3',5'-bisphosphate was obtained as the ammonium salt after evaporation of fractions from the ion-exchange column {of example 30} (0.35 mmol, 20% yield).

This was characterised by the pattern of spots observed on TLC after incubation with nuclease P1 (Boehringer Mannheim), alkaline phosphatase (Boehringer Mannheim), 3-nucleotidase (Sigma) and 5'-nucleotidase. Alkaline phosphatase totally cleaved 9-(2-Deoxy-2-fluoro- β -D-ribofuranosyl)adenine-3',3'-bisphosphate to the parent nucleoside; nuclease P1 and 3-nucleotidase cleaved it to 9-(2-Deoxy-2-fluoro- β -D-ribofuranosyl)adenine-5'-monophosphate and 5'-nucleotidase did not cleave it. These results were consistent with what is known for these enzymes and other known nucleoside phosphate analogs.

UV spectra: in 400 mM ammonium phosphate, pH 5.5 max at 259 nm.

45 Example 30

2.6-Diamino-9-(2-deoxy-2-fluoro-3,5-di-O-pivaloyl- β -D-ribofuranosyl)-9H-purine and 2.6-diamino-9-(2-deoxy-2-fluoro-5-O-pivaloyl- β -D-ribofuranosyl)-9H-purine

50 To a solution of 2,6-diamino-9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-9H-purine (100 mg, 0.35 mmol) in a solution of DMF (3ml) and Et_3N (0.3 ml) was added trimethylacetic anhydride (78 μl) and the solution stirred at room temperature overnight. A further 80 μl of trimethylacetic anhydride was then added and stirring continued for 3 days. The mixture was then quenched with MeOH, evaporated in vacuo and chromatographed over flash SiO_2 , eluting w/ CHCl_3 , $\text{CHCl}_3/\text{MeOH}$ (30:1), (20:1), (10:1), (6:1), (4:1) and finally (3:1). This gave the bis-ester (74 mg) as a colourless semi-solid after trituration with ether.

55 mp. 143-145°C (dec.)

High resolution mass spectrum (E.I.):

Found: 368,1612 $\text{C}_{15}\text{H}_{21}\text{FN}_6\text{O}_4$ requires 368.1608.

Collection and evaporation of the appropriate fractions also gave the mono-5'-pivalate ester (16 mg).
mp. 123-125°C.

High resolution mass spectrum (E.I.) :

Found: 452.2181 C₂₀ H₂₉ FN₆ O₅ required 452.2183.

5

Example 31

2-Amino-6-benzylamino-9-(2'-deoxy-2'-fluoro-β-D-ribofuranosyl)-9H-purine

10 2-Amino-6-benzylaminopurine (0.2 g, 0.83 mmole) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil (0.244 g, 1 mmole) were combined with 10 ml of 10 mM potassium phosphate buffer, pH 6.8. 8200 units of thymidine phosphorylase and 7850 units of purine nucleoside phosphorylase (Krenitsky, *et al* *Biochemistry*, **20**, 3615, 1981 and US Patent 4,381,444) were added and the mixture stirred at 45° C for 40 hours. Product isolation was accomplished by adding methanol (15 ml), removal of the solids by filtration, and evaporating the methanol in the presence of 10 ml silica gel.
15 The dry gel was applied to the top of a column of silica (5 x 23 cm) and the product eluted with chloroform : methanol (99:1). The fractions containing only the product were combined the solvent removed under vacuum resulting in 0.087 g of 2-amino-6-benzylamino-9-(2'-deoxy-2'-fluoro-β-D-ribofuranosyl)-9H-purine.

19 ¹H-NMR (200 mHz) : δ 7.95 (s, 1 H, H₈), 7.85 (b, 1H, NH), 7.2-7.5(m, 5H, phenyl), 6.04 (dd, 1H, H₁, *J*_{1,2}=16.4 Hz, *J*=3.1 Hz) 5.93 (b, 2H, NH₂), 5.64(d, 1H, OH, *J*=5.9 Hz), 5.44, 5.17 (dt, 1H, H₂), 5.26 (t, 1H, OH₅), 4.64 (b, 2H, CH₂), 4.38 (m, 1H H₃), 3.9(b, 1H, H₄), 3.5-3.75 (m, 2H, H₅).

20 Reduction of the above compound may be accomplished according to the procedures of V.du Vigneaud and O.K. Behrens *J.Biol.Chem.* **117**, 27 (1937).

Example 32

25 2-Amino-6-benzylthio-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine

2-Amino-6-benzylthiopurine (Sigma Chemical Company; 0.8 g, 3.1 mmoles) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil (0.4 g, 1.7 mmoles) which may be prepared according to J.F.Codington *et al.*, (*J.Org Chem.* **29**:558, 1964) were suspended in 20 ml of 10 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. Thymidine phosphorylase (2,640 I.U.) and purine nucleoside phosphorylase (4,360 I.U.) (T.A.Krenitsky *et al.*, *Biochemistry* **20**:3615, 1981 and U.S. Patent 4,381,344) were added and the suspension stirred at 37°C. On day 21, the reaction was diluted to 150 ml with 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide and 4,000 I.U. thymidine phosphorylase and 6,500 I.U. purine nucleoside phosphorylase were added. On day 43, the reaction was diluted to 250 ml with water and 2,000 I.U. thymidine phosphorylase and 3,250 I.U. purine nucleoside phosphorylase were added. On day 69, the pH of the reaction was adjusted to 7.1 with KOH. On day 77, the reaction was evaporated. The residue was dissolved in hot methanol/water and applied to a 2.5 x 7 cm column of anion exchange resin (Bio-Rad AGIX2-hydroxide form) and the product was eluted with methanol/water (9/1). Product containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in acetonitrile/water (49/1) and applied to a 2.5 x 20 cm column of silica gel 60 (EM Science). The product was eluted with acetonitrile/water (49/1). Product containing fractions were combined and the solvent removed under vacuum. The residue was suspended in water and lyophilization yielded 0.201 g of title compound that analyzed as a 0.1 hydrate.

mp. 180°C

UV λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1N HCl, 322.5 (11.8), 250 (10.6); pH 7, 311.5 (14.2), 247 (14.3); 0.1N NaOH, 312 (14.0), 247 (13.5).

Anal. Calcd. for C ₁₇ H ₁₈ FN ₅ O ₃ S.0.1H ₂ O					
Calcd.:	C, 51.93;	H, 4.66;	N 17.81;	F, 4.83	S, 8.15
Found:	C, 51.96;	H, 4.66;	N 17.86;	F, 4.68	S, 8.15

50 1H-NMR (300 mHz, Me₂SO-d₆): δ 8.19 (s, 1H, H-8), 7.47(apparent d, 2H, Ar, *J*=7.6Hz), 7.28(m, 3H, Ar), 6.74(bs, 2H, 2-NH₂), 6.10(dd, 1H, H-1', *J*_{F, 1'}=16.6Hz, *J*-2.4Hz), 5.68(d, 1H, 3'-OH, *J*=6.4Hz), 5.32(ddd, 1H, H-2', *J*_{F, 2'}=53.0 Hz, *J*_{1,2}=2.4 Hz, *J*_{2,3}=4.4 Hz, 5.15(t, 1H, 5'-OH, *J*=6.0 Hz, 4.55(ab quartet, 2H, 6-SCH₂, geminal *J*=-13.7 Hz), 4.41(m, 1H, H-3'), 3.94(m, 1H, H-4'), 3.74(m, 1H, H_α-5'), 3.58 (m, 1H, H_β-5').

Example 332,6-Diamino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine

5 2,6-Diaminopurine (Pacific Chemical Laboratories, 2.0 g, 12.7 mmoles) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl) uracil (0.8 g; 3.3 mmoles) which may be prepared according to J.F. Codington *et al.*, (*J. Org. Chem.* 29:558, 1964) were suspended to 500 ml of 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. Thymidine phosphorylase (41,700 I.U.) and purine nucleoside phosphorylase (83,300 I.U.) (T.A. Krenitsky *et al.*, *Biochemistry* 20:3615, 1981 and U.S. Patent 4,381,344) which had been absorbed onto 10.5 g of DEAE-cellulose (25 ml)

10 were added and the suspension shaken at 37°C. After 24 hours 2.0 g of 1,6-diaminopurine was added and the temperature increased to 50°C. After another 24 hours the reaction was filtered. The filter cake was washed with water and the filtrates combined and the solvent removed under vacuum. The residue was dissolved in hot water and the pH adjusted to pH 9.4 with NH₄OH. The solution was applied to a 2.5 x 13 cm column of anion exchange resin (Bio-Rad AGIX2-hydroxide form). After washing the column with water, the product was eluted with methanol/water (9/1). Product

15 containing fractions were combined and the solvent removed under vacuum. The residue was treated as above. Product containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in water and lyophilization yielded 0.89 g of title compound that analyzed as a 0.5 hydrate.

mp. 125-127°C

UV λ_{max} nm ($\epsilon \times 10^{-3}$): pH 7, 279.5 (9.52, 256 (9.06);

25 Structure further confirmed by ¹H-NMR.

Example 342-Amino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine

30 2-Aminopurine (Pacific Chemical Laboratories, 3.0 g, 22.2 mmoles) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl) uracil (0.5 g; 2.0 mmoles) which may be prepared according to J.F. Codington *et al.*, (*J. Org. Chem.* 29:558, 1964) were suspended in 25 ml of 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. Thymidine phosphorylase (41,700 I.U.) and purine nucleoside phosphorylase (83,300 I.U.) (T.A. Krenitsky *et al.*, *Biochemistry* 20:3615, 1981 and U.S. Patent 4,381,344) which had been absorbed onto 10.5 g of DEAE-cellulose (25 ml) were added and the suspension shaken at 37°C. After 24 hours 3.0 g of 2,6-diaminopurine was added and the temperature increased to 50°C. After another 24 hours the reaction was filtered. The filter cake was washed with water and the filtrates combined and the solvent removed under vacuum. The residue was suspended in water and filtered. The filter cake was extracted with water (25°C) until no product remained in the filter cake. The filtrates were combined, the pH was adjusted to pH 9.4 with NH₄OH, and the solution applied to a 2.5 x 20 cm column of anion exchange resin (Bio-Rad AGIX2-hydroxide form). After washing the column with water, the product was eluted with methanol/water (9/1). Product containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in chloroform/methanol/water (75:25:4) and applied to a 5 x 25 cm column of silica gel 60. The product was eluted with chloroform/methanol/water (75:25:4). Product containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in hot water and filtered through a 0.22 µm-pore membrane filter. Lyophilization of the filtrate yielded 0.50 g of title compound that analyzed as a 0.5 hydrate.

mp. 153-155°C

UV λ_{max} nm ($\epsilon \times 10^{-3}$): pH 7, 304 (6.35), 243.5 (5.95)

55 Structure further confirmed by ¹H-NMR.

Anal. Calcd. for C ₁₀ H ₁₃ FN ₆ O ₃ -0.5H ₂ O				
Calcd.:	C, 40.96;	H, 4.81;	N 28.66;	F, 6.48
Found:	C, 41.03;	H, 4.80;	N 28.69;	F, 6.50

Anal. Calcd. for C ₁₀ H ₁₂ FN ₅ O ₃ -0.5H ₂ O				
Calcd.:	C, 43.17;	H, 4.71;	N 25.17;	F, 6.83
Found:	C, 43.08;	H, 4.74;	N 25.11;	F, 6.89

Example 352-Amino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-6-methoxy-9H-purine

5 2-Amino-6-methoxypurine (2.0 g, 12 mmoles) which may be prepared according to R.W.Balsiger and J.A.Montgomery (*J.Org.Chem.* 20:1573, 1960) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil (0.88 g; 3.6 mmoles) which may be prepared according to J.F. Codington *et al.*, (*J.Org.Chem.* 29:558, 1964) were suspended in 250 ml of 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) potassium azide. The pH of the suspension was adjusted to 7.0 with KOH. Thymidine phosphorylase (41,700 I.U.) and purine nucleoside phosphorylase (83,300 I.U.) (T.A.Krentitsky *et al.*, *Biochemistry* 20:3615, 1981 and U.S. Patent 4,381,344) which had been absorbed onto 10.5 g of DEAE-cellulose (25 ml) were added and the suspension shaken at 37°C. After 24 hours 1.0 g of 2-amino-6-methoxypurine and 250 ml of the above buffer were added and the temperature increased to 50°C. After another 24 hours the reaction was filtered. The filter cake was washed with water and the filtrates combined and the solvent removed under vacuum. The residue was dissolved in warm water and the pH adjusted to pH 9.4 with NH₄OH. The solution was applied to a 2.5 x 15 cm column of anion exchange resin (Bio-Rad AG1X2-hydroxide form). After washing the column with water, the product was eluted with methanol/water (9/1). Product containing fractions were combined and the solvent removed under vacuum. The residue was dissolved in warm water/methanol and filtered through a 0.22 μm-pore nylon membrane filter. Lyophilization of the filtrate yielded 0.91 g of title compound that analyzed as a 0.5 hydrate.

10 mp. 200°C

15 UV λ_{max} nm ($\epsilon \times 10^{-3}$): pH7, 279.5 (9.24), 247.5 (9.99).

Anal. Calcd. for C ₁₁ H ₁₄ FN ₅ O ₄ -0.5H ₂ O				
Calcd.:	C, 42.86;	H, 4.90;	N 22.72;	F, 6.16
Found:	C, 42.93;	H, 4.90;	N 22.73;	F, 6.15

20 Structure further confirmed by ¹H-NMR.

Example 362-Amino-6-benzyloxy-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine

30 2-Amino-6-benzyloxypurine (2.5 g, 10.3 mmole) and 2'-deoxy-2'-fluorouridine (2.64 g, 10.8 mmole) were combined with 50 ml of 10 mM potassium phosphate buffer, pH 6.8. 8000 units of thymidine phosphorylase and 21600 units of 35 purine nucleoside phosphorylase were added and the mixture stirred at 45°C. After five days an additional 16000 units of thymidine phosphorylase and 21600 units of purine nucleoside phosphorylase were added and the contents of the reaction stirred for 48 hours at 45°C. The majority of the product was contained in the precipitate which was removed by filtration and dissolved in methanol. Product was isolated by chromatography on silica with ethyl acetate:chloroform:methanol (8:1:1) as the mobile phase. Fractions containing only the product were combined and the solvent was 40 evaporated to give 1.44 g of 2-amino-6-benzyloxy-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine.

¹H-NMR (200 mHz in DMSO): δ 8.11 (s, 1H, H₈), 7.3-7.5 (m, 5H, phenyl), 6.59 (b, 2H, NH₂), 6.09 (dd, 1H, H₁, J_{F,1}=16.5 Hz, J=2.6 Hz), 5.66 (d, 1H, OH, J=6.1 Hz), 5.48 (s, 2H, CH₂), 5.25, 5.53 (bd, 1H, H_{2'}), 5.20 (m, 1H, OH_{5'}), 4.3-4.5 (m, 1H, H_{3'}), 3.9 (b, 1H, H_{4'}), 3.5-3.8 (m, 2H, H_{5'}).

45 The generation of the compound of Example 7 from 2-amino-6-benzyloxy-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine is accomplished by the procedure of El Amin *et al.*, *J.Org.Chem.*, 44, 3442 (1979).

Pharmaceutical Formulations

50 In the following formulation Examples, the "Active Ingredient" may be any compound of formula (I) or a pharmaceutically acceptable salt thereof, for example compounds of Examples 6, 7, 9 and 12.

Example 37Tablet Formulations

55 The following formulations A, B and C are prepared by wet granulation of the ingredients with a solution of povidone, followed by addition of magnesium stearate and compression.

5

10

<u>Formulation A</u>	<u>mg/tablet</u>	<u>mg/tablet</u>
(a) Active ingredient	250	250
(b) Lactose B.P.	210	26
(c) Povidone B.P.	15	9
(d) Sodium Starch Glycollate	20	12
(e) Magnesium Stearate	5	3
	<u>500</u>	<u>300</u>

15

20

35

<u>Formulation B</u>	<u>mg/tablet</u>	<u>mg/tablet</u>
(a) Active ingredient	250	250
(b) Lactose	150	-
(c) Avicel PH 101	60	26
(d) Povidone B.P.	15	9
(e) Sodium Starch Glycollate	20	12
(f) Magnesium Stearate	5	3
	<u>500</u>	<u>300</u>

25

30

35

<u>Formulation C</u>	<u>mg/tablet</u>
Active ingredient	100
Lactose	200
Starch	50
Povidone	5
Magnesium stearate	4
	<u>359</u>

The following formulations, D and E, are prepared by direct compression of the admixed ingredients.

40

45

<u>Formulation D</u>	<u>mg/capsule</u>
Active Ingredient	250
Pregelatinised Starch NF15	150
	<u>400</u>

50

55

<u>Formulation E</u>	<u>mg/capsule</u>
Active Ingredient	250
Lactose	150
Avicel	100
	<u>500</u>

Formulation F (Controlled Release Formulation)

The formulation is prepared by wet granulation of the following ingredients with a solution of povidone followed by

the addition of magnesium stearate and compression.

		<u>mg/tablet</u>
5	(a) Active Ingredient	500
	(b) Hydroxypropylmethylcellulose (Methocel K4M Premium)	112
	(c) Lactose B.P.	53
	(d) Povidone B.P.C.	28
10	(e) Magnesium Stearate	7
		<u>700</u>

Drug release takes place over a period of about 6-8 hours and is complete after 12 hours.

Example 38

Capsule Formulations

Formulation A

20 A capsule formulation is prepared by admixing the ingredients of Formulation D in Example 4 above and filling into a two-part hard gelatin capsule.

<u>Formulation B</u>	
	<u>mg/capsule</u>
25	(a) Active ingredient 250
	(b) Lactose B.P. 143
	(c) Sodium Starch Glycollate 25
30	(d) Magnesium Stearate 2
	<u>420</u>

Capsules are prepared by admixing the above ingredients and filling into a two-part hard gelatin capsule.

<u>Formulation C</u>	
	<u>mg/capsule</u>
35	(a) Active ingredient 250
40	(b) Macrogol 4000 BP 350
	<u>600</u>

Capsules are prepared by melting the Macrogol 4000 BP, dispersing the active ingredient in the melt and filling the melt into a two-part hard gelatin capsule.

<u>Formulation D</u>	
	<u>mg/capsule</u>
45	Active ingredient 250
	Lecithin 100
50	Arachis Oil 100
	<u>450</u>

55 Capsules are prepared by dispersing the active ingredient in the lecithin and arachis oil and filling the dispersion into soft, elastic gelatin capsules.

Formulation E (Controlled Release Capsule)

The following controlled release capsule formulation is prepared by extruding ingredients (a), (b) and (c) using an extruder, followed by spheronisation of the extrudate and drying. The dried pellets are then coated with release-controlling membrane (d) and filled into a two-piece, hard gelatin capsule.

	<u>mg/capsule</u>
(a) Active Ingredient	250
(b) Microcrystalline Cellulose	125
(c) Lactose BP	125
(d) Ethyl Cellulose	13
	<u>513</u>

Example 39Injectable Formulation

<u>Formulation A</u>	
Active ingredient	0.200g
Hydrochloric acid solution, 0.1M	q.s. to pH 4.0 to 7.0
Sodium hydroxide solution, 0.1M	q.s. to pH 4.0 to 7.0
Sterile water	q.s. to 10ml

The active ingredient is dissolved in most of the water (35°-40°C) and the pH adjusted to between 4.0 and 7.0 with the hydrochloric acid or the sodium hydroxide as appropriate. The batch is then made up to volume with the water and filtered through a sterile micropore filter into a sterile 10ml amber glass vial (type 1) and sealed with sterile closures and overseals.

<u>Formulation B</u>	
Active ingredient	0.125 g
Sterile, pyrogen-free, pH 7 phosphate buffer, q.s. to 25 ml	

Example 40Intramuscular injection

Active Ingredient	0.20 g
Benzyl Alcohol	0.10 g
Glycofurol 75	1.45 g
Water for Injection q.s. to	3.00 ml

The active ingredient is dissolved in the glycofurol. The benzyl alcohol is then added and dissolved, and water added to 3 ml. The mixture is then filtered through a sterile micropore filter and sealed in sterile 3 ml amber glass vials (type 1).

Example 41Syrup

5

Active ingredient	0.25 g
Sorbitol Solution	1.50 g
Glycerol	2.00 g
Sodium Benzoate	0.005 g
Flavour, Peach 17.42.3169	0.0125 ml
Purified Water q.s. to	5.00 ml

10 The active ingredient is dissolved in a mixture of the glycerol and most of the purified water. An aqueous solution of the sodium benzoate is then added to the solution, followed by addition of the sorbitol solution and finally the flavour. The volume is made up with purified water and mixed well.

Example 42

15

Powder Capsules for Inhalation

20

Active Ingredient (0.5-.70µm powder)	4 mg
Lactose (30-90µm powder)	46.0 mg

25 The powders were mixed until homogeneous and filled into suitably sized hard gelatin capsules, 50 mg of mixture per capsule.

25

Example 43

30

Inhalation Aerosol

35

Active Ingredient (0.5-7.0 µm powder)	200 mg
Sorbitan Trioleate	100 mg
Saccharin Sodium (0.5-7.0 µm powder)	5 mg
Methanol	2 mg
Trichlorofluoromethane	4.2 g
Dichlorodifluoromethane to	10.0 ml

40

The Sorbitan Trioleate and Menthol were dissolved in the Trichlorofluoromethane. The Saccharin Sodium and active ingredient were dispersed in the mixture which was then transferred to a suitable aerosol canister and the Dichlorofluoromethane injected through the valve system. This composition provides 2 mg of active ingredient in each 100 µl dose.

45

Antiviral Activity
Influenza A and B strains were assayed in monolayers of primary chick embryo(ce) cells in multiwell trays. Activity of compounds was determined in the yield reduction or in the plaque reduction assay, in which a cell monolayer was infected with a suspension of influenza virus and then overlaid with liquid medium (yield reduction) or with nutrient agarose in the form of a gel to ensure that there is no spread of virus throughout the culture (plaque reduction). A range of concentration of compound of known molarity was incorporated into the medium/nutrient agarose overlay. Yield of virus or plaque numbers of each concentration are expressed as a percentage of the control and a dose response curve is drawn. From this curve the 50% inhibitory concentration (IC_{50}) is estimated. Respiratory Syncytial Virus (RSV) is assayed in BS-C-1 cells (African Green Monkey kidney cells) by a similar plaque/foci reduction assay. The results are presented in Tables I and II below.

55

In vivo inhibitory activity was assessed for Influenza A and B strains in a mouse intranasal/lung model. Mice were infected with virus by aerosol in an enclosed box and then treated with test compounds at various times after infection by various routes, including oral, intraperitoneally and by aerosol. The mice were sacrificed after 24 hours and 10% lung suspensions prepared and titrated for presence of virus. Results were recorded as a reduction in virus growth compared

with untreated controls.

References

5 Appleyard, G and Maber, H.B, Plaque Formation by Influenza Vi-ruses in the Presence of Trypsin. *J.Gen.Viro*l.25, 351-357. (1974). Collins, P. and Bauer, D.J, Relative Potencies of Anti-Herpes Compounds. *Ann.N.Y.Acad. Sci.*284, 49-59, (1977). Hayden, F.G, Cotek, M. and Douglas, R.G., Plaque Inhibition Assay for Drug Susceptibility Testing of Influenza Viruses. *Antimicro Agents and Chemo*.17. 865-870. (1980). Tisdale, M. and Bauer, D.J. A comparison of test methods in influenza chemotherapy. *J. Antimicrob. Chemother.* 1 suppl. 55-62 (1975).

10

Table I

Ex. No.	Compound	Anti-influenza Activity	
		R1 R2 X Y	CE cells IC ₅₀ , μM
15	6 OH OH NH ₂ NH ₂		0.6
	8 OH OH NH ₂ H		4
	9 OH OH H NH ₂		-
	12 OH OH OMe NH ₂		0.4
20			+(2.1)
			+(2.3)
			+(1.6)
			+(1.9)

20

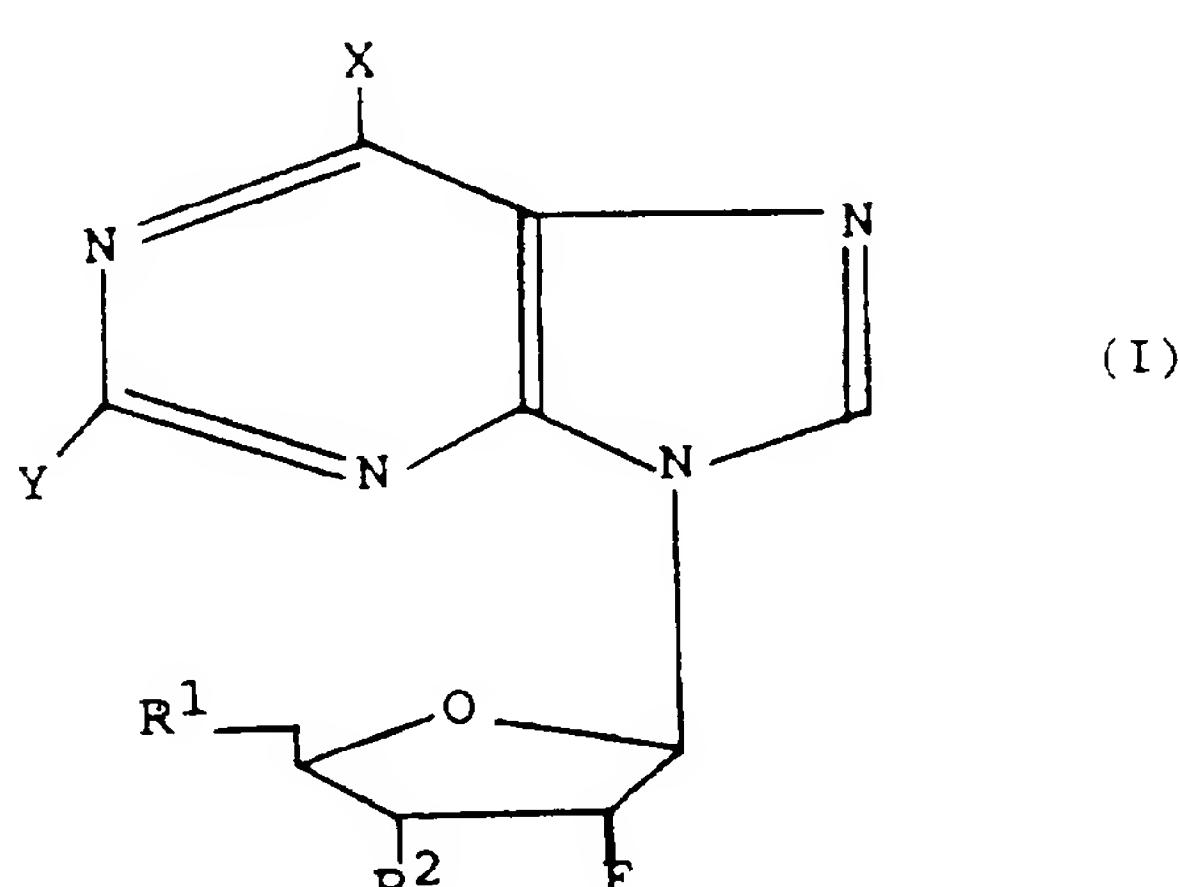
Table I

Ex. No.	Compound	Anti-respiratory syncytial virus
25	12 OH OH OMe NH ₂	BS-C-1-cells IC ₅₀ μM 6.3

30 **Claims**

Claims for the following Contracting States : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE

35 1. A compound of formula (I)



wherein Y is H or NH₂;

55 X is a group -NR³R⁴ in which R³ and R⁴, which may be the same or different, each represent hydrogen;

C₁₋₆ alkyl, C₂₋₆ alkenyl, C₃₋₇ cycloalkyl, each group optionally being substituted by one or more halogens, or X is a group Z-R⁵ in which Z is oxygen or sulphur and R⁵ has the same definition as R³, or X is halogen or hydrogen; and

R^1 and R^2 , which may be the same or different, each represent:

- a hydroxy group;
- a group $-OCOR^6H$ where R^6 is a divalent group which is straight or branched chain C_{1-6} alkylene, C_{2-6} alkenylene or C_{3-7} cycloalkylene, each being optionally substituted by one or more hydroxy groups;
- a group $-OCO_2R^7H$ where R^7 is a covalent bond or is as defined for R^6 ;
- a group $-OCOR^6-COOR^8$ where R^6 is as defined above and R^8 is selected from hydrogen, or straight or branched chain C_{1-6} alkyl or C_{1-6} alkenyl each being optionally substituted by one or more hydroxy groups;
- a group $-OCOR^7-Z-Ar$ where R^7 is as defined above, Z is either a covalent bond or oxygen and Ar is an aromatic ring unsubstituted or substituted by one or more halogen, C_{1-6} alkyl or C_{1-6} alkoxy;
- a group $-OR^6H$ where R^6 is as defined above;
- a group $-OR^6-Z-Ar$ where R^6 , Z and Ar are as defined above;
- a group $-OCOCHR^9NR^{10}R^{11}$ where R^{10} and R^{11} are as defined for R^8 , and R^9 is
 - hydrogen,
 - straight or branched chain C_{1-4} alkyl optionally substituted by one or more hydroxy, mercapto, C_{1-3} alkoxy or C_{1-3} alkylthio groups
 - a group $R^{12}-A$ where R^{12} is a C_{1-4} alkylene group optionally substituted by one or more hydroxy groups and A is a group $-COOH$, $-CONH_2$, $-NH_2$ or $-NH-C(NH)NH_2$ or A is a 4- to 11-membered aromatic or non aromatic cyclic or heterocyclic ring system containing 3 to 10 carbon atoms and 0, 1, 2 or 3 ring nitrogen atoms, the ring carbon atoms and/or nitrogen atoms being optionally substituted by one or more hydroxy groups;
- a group $-OCO-R^{13}$ where R^{13} is a 4- to 7-membered heterocyclic ring containing 3 to 6 carbon atoms and 0, 1 or 2 nitrogen atoms, the ring carbon atoms and/or nitrogen atoms being optionally substituted by one or more hydroxy groups; or
- a mono-, di- or tri-phosphate ester group or a pharmaceutically acceptable salt thereof; other than compounds of formula (I) in which R^1 and R^2 are each hydroxy or R^1 is a mono-, di- or tri-phosphate 5'-ester and R^2 is hydroxy and either (a) X is OH and Y is NH_2 or H ; or (b) X is NH_2 and Y is H .

2. A compound according to Claim 1 characterised in that R^1 and R^2 are either both OH , or R^1 is a monophosphate and R^2 is OH , Y is H or NH_2 and X is a group $-NR^3R^4$ in which R^3 and R^4 , which may be the same or different, represent H , or C_{1-6} alkyl, provided R^3 and R^4 are not both hydrogen, or X is a group $Z-R^5$ in which Z is O or S and R^5 is C_{1-6} alkyl, or X is halogen or hydrogen, or a pharmaceutically acceptable salt thereof.

3. A compound according to Claim 1 or 2 characterised in that R^1 and R^2 are both OH , Y is NH_2 and X is H , NH_2 or $Z-R^5$ where Z is O and R^5 is C_{1-6} alkyl, or a pharmaceutically acceptable salt thereof.

4. 2,6-Diamino-9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-9H-purine and pharmaceutically acceptable salts thereof.

5. 2-Amino-9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-9H-purine and pharmaceutically acceptable salts thereof.

6. 2-Amino-9-(2-deoxy-2-fluoro- β -D-ribofuranosyl)-6-methoxy-9H-purine and pharmaceutically acceptable salts thereof.

7. A compound according to any of claims 1 to 6 for use in medical therapy.

8. Use of a compound as defined in any of Claims 1 to 6 in the manufacture of a medicament for the treatment or prophylaxis of an infection.

9. Use of a compound as claimed in claim 8 wherein the infection is a viral infection.

10. Use of a compound as claimed in claim 8 wherein the infection is a protozoal infection.

5 11. Use of a compound as claimed in claim 8 wherein the viral infection is an influenza virus infection.

12. Use of compound as claimed in claim 8 wherein the viral infection is an respiratory syncytial virus infection.

10 13. Use of a compound as claimed in claim 8 wherein the protozoal infection is a Trichomonas vaginalis infection.

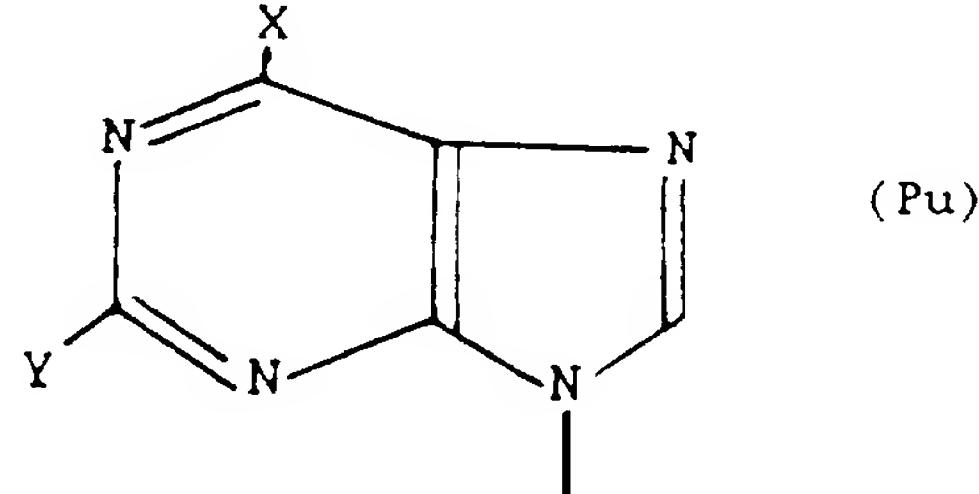
14. Use of a compound as claims in claim 8 wherein the protozoal infection is Giardia lamblia infection.

15 15. A pharmaceutical formulation comprising a compound according to any of claims 1 to 7, together with a pharmaceutically acceptable carrier therefor.

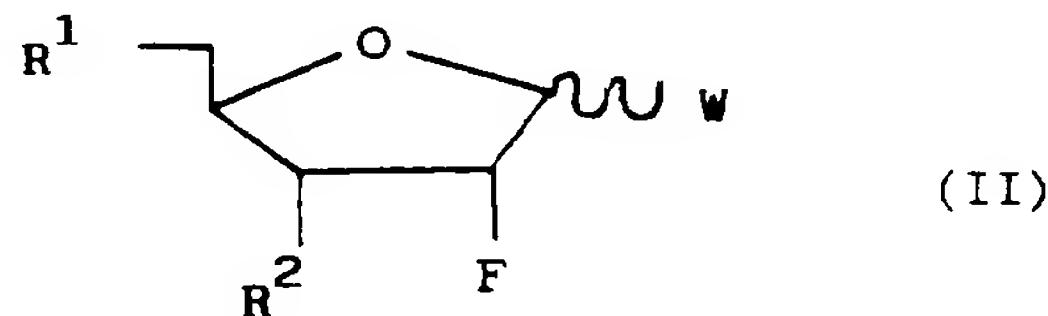
16. A pharmaceutical formulation as claimed in Claim 15 adapted for oral or nasal administration.

20 17. A process for the preparation of a compound of formula (I) (as claimed in Claim 1 to 6 or a pharmaceutically acceptable salt thereof which comprises:

(A) reacting a purine base of formula Pu wherein PuH represents the purine residue:-



(wherein X and Y are as described above), or a salt thereof, with a compound of formula (II)



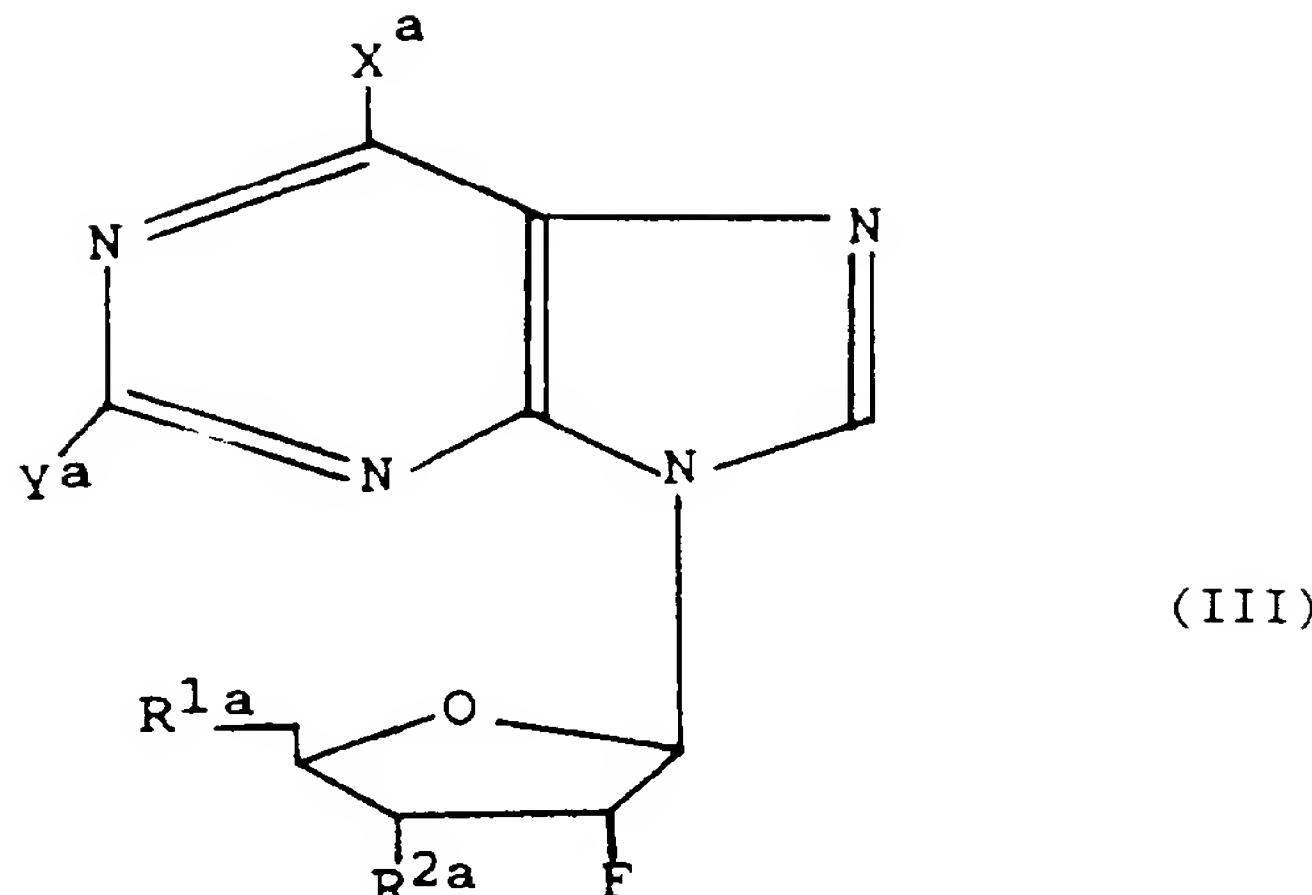
40 wherein R¹ and R² are as hereinbefore defined, and W is either a phosphate ester or salt thereof or a purine or pyrimidine moiety (other than Pu) to form a compound of formula (I);

(B) reacting a compound of formula (III)

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20 (wherein X^a, Y^a, R^{1a} and R^{2a} respectively represent the groups X, Y, R¹ and R² as defined above or a precursor, e.g. protected, form of such a group providing at least one of X, Y, R¹ and R² represents a precursor form) with an agent serving to convert the said precursor form of the group(s) to the desired group(s); and thereafter or simultaneously therewith effecting one or more of the following optional conversions:

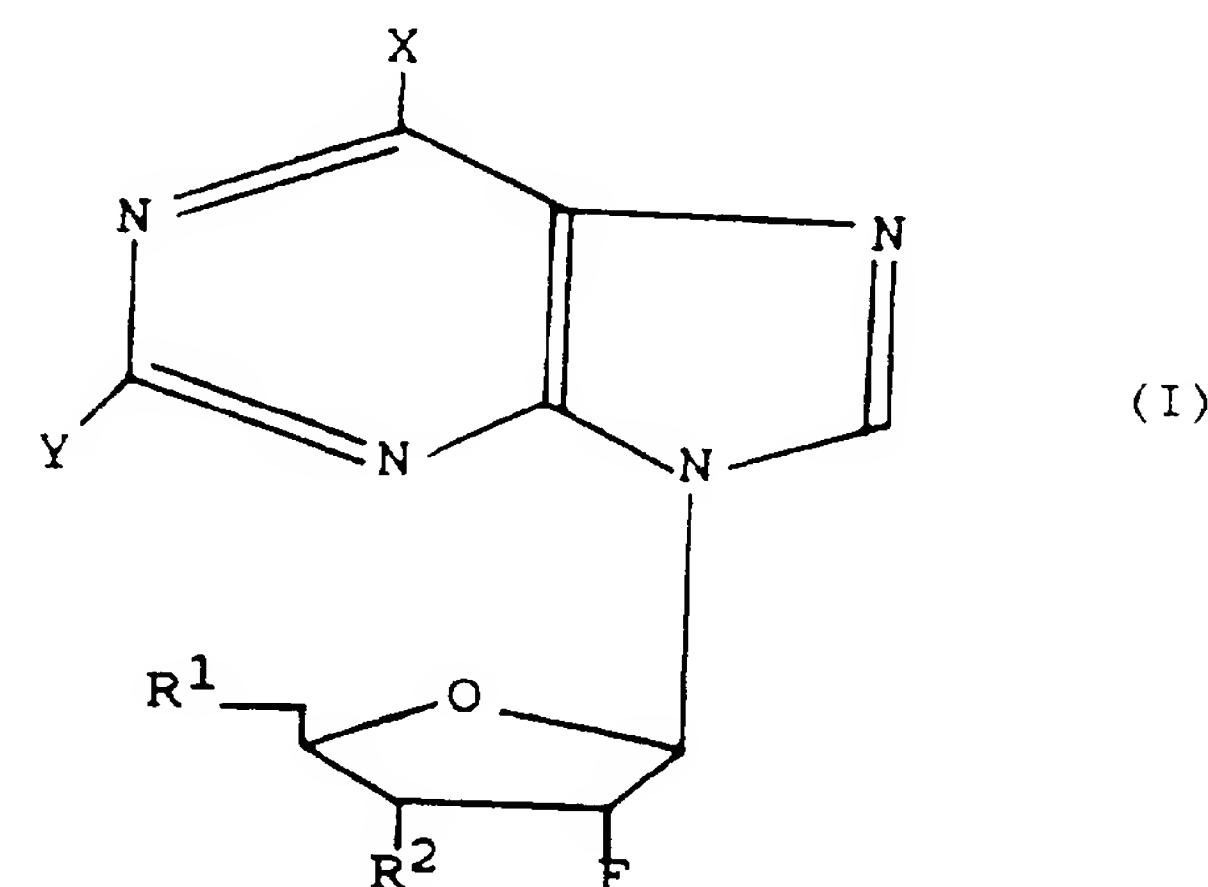
25 (i) reacting a compound of formula (I) wherein at least one of R¹ and R² is a hydroxy group with an appropriate agent serving to convert the said hydroxy group to an alternative group represented by R¹ and/or R².

(ii) reacting a compound of formula (I) wherein R¹ and/or R² are not hydroxy with an agent serving to convert the said R¹ and/or R² group to a hydroxy group.

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Claims for the following Contracting States : ES, GR

35 1. A process for the preparation of a compound of formula (I)



wherein Y is H or NH₂;

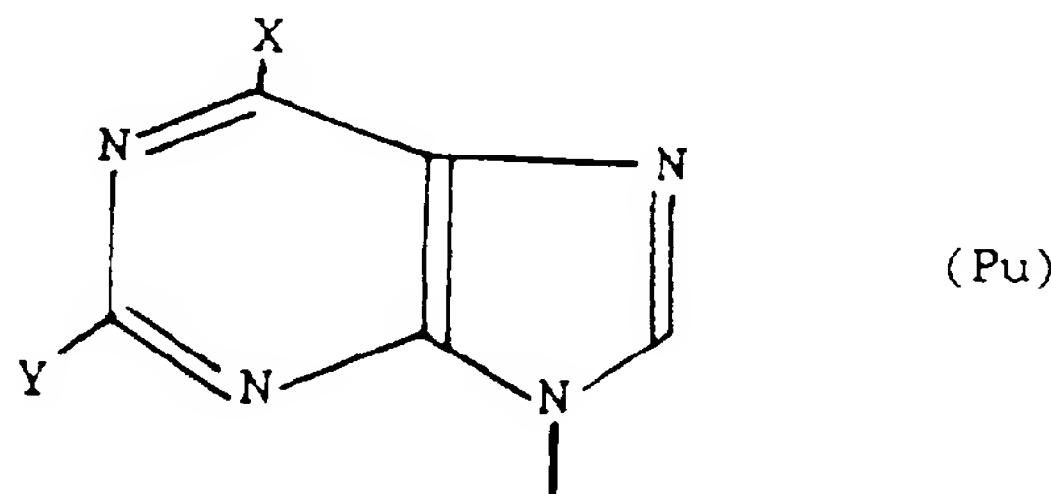
X is a group -NR³R⁴ in which R³ and R⁴, which may be the same or different, each represent hydrogen;

55 C₁₋₆ alkyl, C₂₋₆ alkenyl, C₃₋₇ cycloalkyl, each group optionally being substituted by one or more halogens, or X is a group Z-R⁵ in which Z is oxygen or sulphur and R⁵ has the same definition as R³, or X is halogen or hydrogen; and

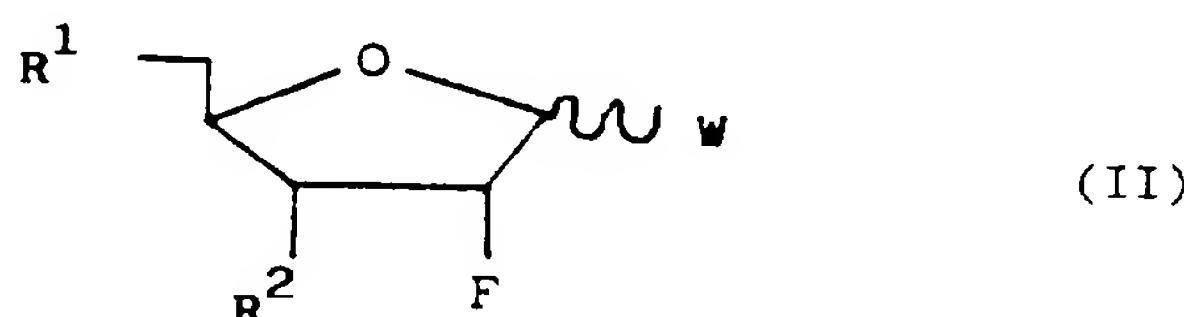
R¹ and R², which may be the same or different, each represent:

- a hydroxy group;
- a group -OCOR⁶H where R⁶ is a divalent group which is straight or branched chain C₁₋₆ alkylene, C₂₋₆ alkenylene, or C₃₋₇ cycloalkylene; each being optionally substituted by one or more hydroxy groups;
- a group -OCO₂R⁷H where R⁷ is a covalent bond or is as defined for R⁶;
- a group -OCOR⁶-COOR⁸ where R⁶ is as defined above and R⁸ is selected from hydrogen, or straight or branched chain C₁₋₆ alkyl or C₁₋₆ alkenyl each being optionally substituted by one or more hydroxy groups;
- a group -OCOR⁷-Z-Ar where R⁷ is as defined above, Z is either a covalent bond or oxygen and Ar is an aromatic ring unsubstituted or substituted by one or more halogen, C₁₋₆ alkyl or C₁₋₆ alkoxy;
- a group -OR⁶H where R⁶ is as defined above;
- a group -OR⁶-Z-Ar where R⁶, Z and Ar are as defined above;
- a group -OCOCHR⁹NR¹⁰R¹¹ where R¹⁰ and R¹¹ are as defined for R⁸, and R⁹ is
 - hydrogen,
 - straight or branched chain C₁₋₄alkyl optionally substituted by one or more hydroxy, mercapto, C₁₋₃ alkoxy or C₁₋₃ alkylthio groups
 - a group R¹²-A where R¹² is a C₁₋₄ alkylene group optionally substituted by one or more hydroxy groups and A is a group -COOH, -CONH₂, -NH₂ or -NH-C(NH)NH₂ or A is a 4- to 11-membered aromatic or non aromatic cyclic or heterocyclic ring system containing 3 to 10 carbon atoms and 0, 1, 2 or 3 ring nitrogen atoms, the ring carbon atoms and/or nitrogen atoms being optionally substituted by one or more hydroxy groups;
- a group -OCO-R¹³ where R¹³ is a 4- to 7-membered heterocyclic ring containing 3 to 6 carbon atoms and 0, 1 or 2 nitrogen atoms, the ring carbon atoms and/or nitrogen atoms being optionally substituted by one or more hydroxy groups; or
- a mono-, di- or tri-phosphate ester group or a pharmaceutically acceptable salt thereof other than compounds of formula (I) in which R¹ and R² are each hydroxy or R¹ is a mono; di- or tri-phosphate 5'-ester and R² is hydroxy or either (a) X is OH and Y is NH₂ or H or (b) X is NH₂ and Y is H, which comprises either:

(A) reacting a purine base of formula PuH wherein Pu represents the purine residue:-



(wherein X and Y are as described above), or a salt thereof, with a compound of formula (II)



wherein R¹ and R² are as hereinbefore defined, and W is either a phosphate ester or a salt thereof or

a purine or pyrimidine moiety (other than Pu) to form a compound of formula (I);

(B) reacting a compound of formula (III)

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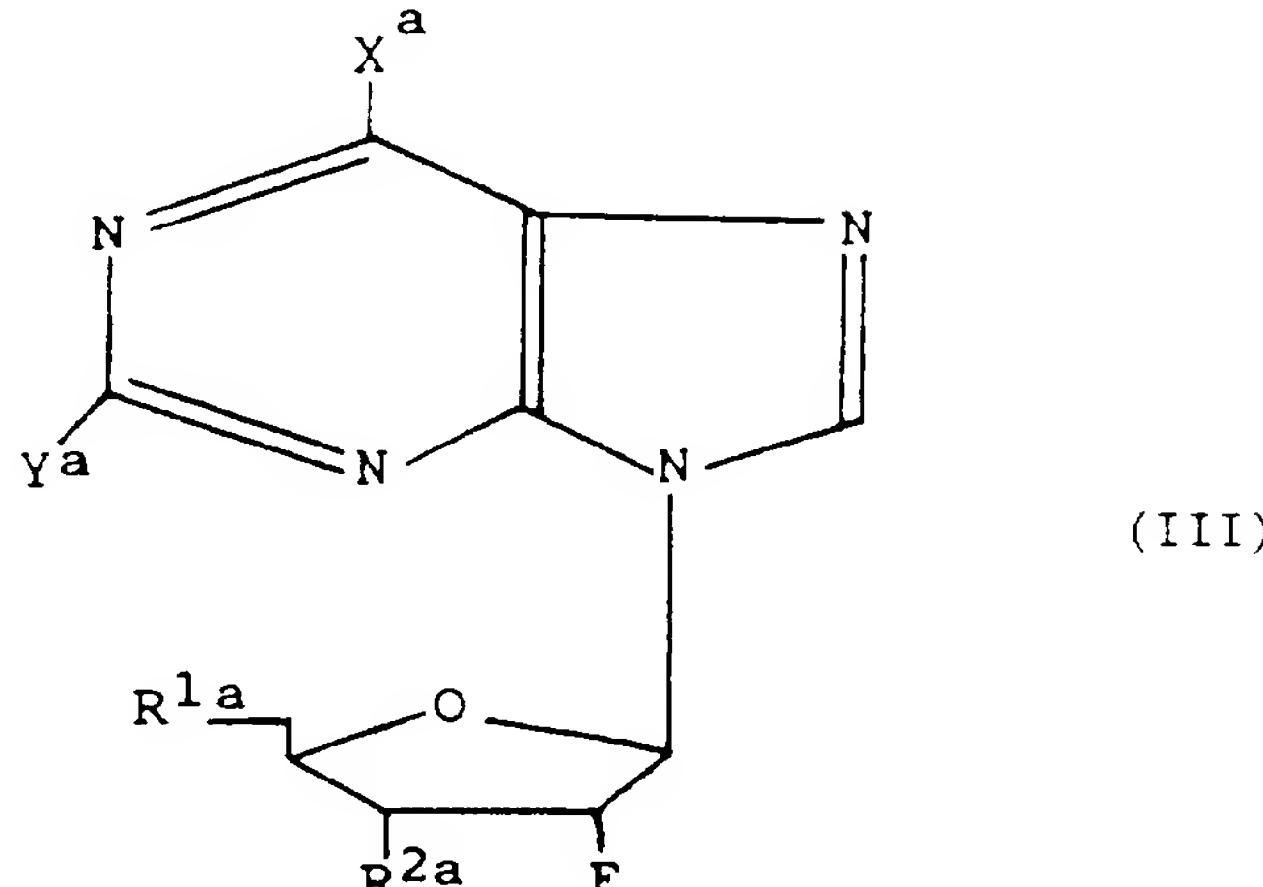
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(wherein X^a, Y^a, R^{1a} and R^{2a} respectively represent the groups X, Y, R¹ and R² as defined above or a precursor, e.g. protected, form of such a group providing at least one of X, Y, R¹ and R² represents a precursor form) with an agent serving to convert the said precursor form of the group(s) to the desired group(s);

and thereafter or simultaneously therewith effecting one or more of the following optional conversions:

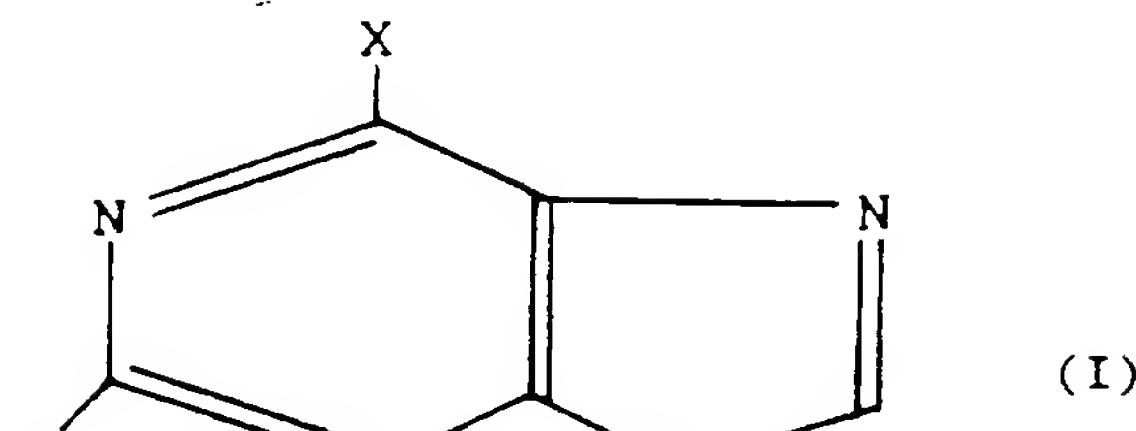
(i) reacting a compound of formula (I) wherein at least one of R¹ and R² is a hydroxy group with an appropriate agent serving to convert the said hydroxy group to an alternative group represented by R¹ and/or R².

(ii) reacting a compound of formula (I) wherein R¹ and/or R² are not hydroxy with an agent serving to convert the said R¹ and/or R² group to a hydroxy group.

2. A process according to Claim 1 for the preparation of a compound of formula (I) wherein R¹ and R² are either both OH, or R¹ is a monophosphate and R² is OH, Y is H or NH₂ and X is a group -NR³R⁴ in which R and R⁴, which may be the same or different, represent H, C₁₋₆ alky, provided R³ and R⁴ are not both hydrogen, or X is a group Z-R⁵ in which Z is O or S and R⁵ is C₁₋₆ alkyl, or X is halogen or hydrogen, or a pharmaceutically acceptable salt thereof.
3. A process according to Claim 1 or 2 for the preparation of a compound of formula (I) wherein R¹ and R² are both OH, Y is NH₂ and X is H, OH, NH₂ or Z-R⁵ where Z is O and R⁵ is C₁₋₆ alkyl, or a pharmaceutically acceptable salt thereof.
4. A process for the preparation of compound 2,6-diamino-9-(2-deoxyfluoro-β-D-ribofuranosyl)-9H-purine and pharmaceutically acceptable salts thereof.
5. A process for the preparation of a compound 2-amino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine and pharmaceutically acceptable salts thereof.
6. A process for the preparation of compound 2-amino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-6-methoxy-9H-purine and pharmaceutically acceptable salts thereof.
7. A process for the preparation of a pharmaceutical formulation containing a compound of formula (I)

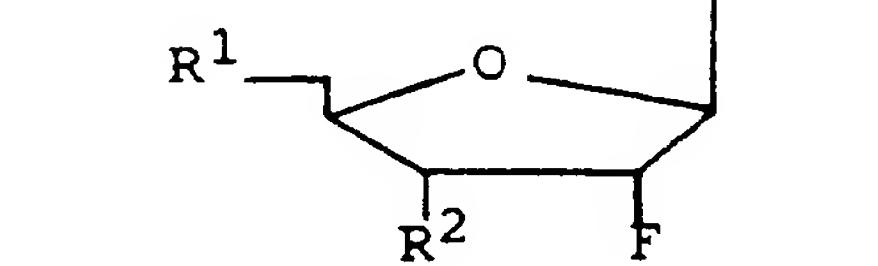
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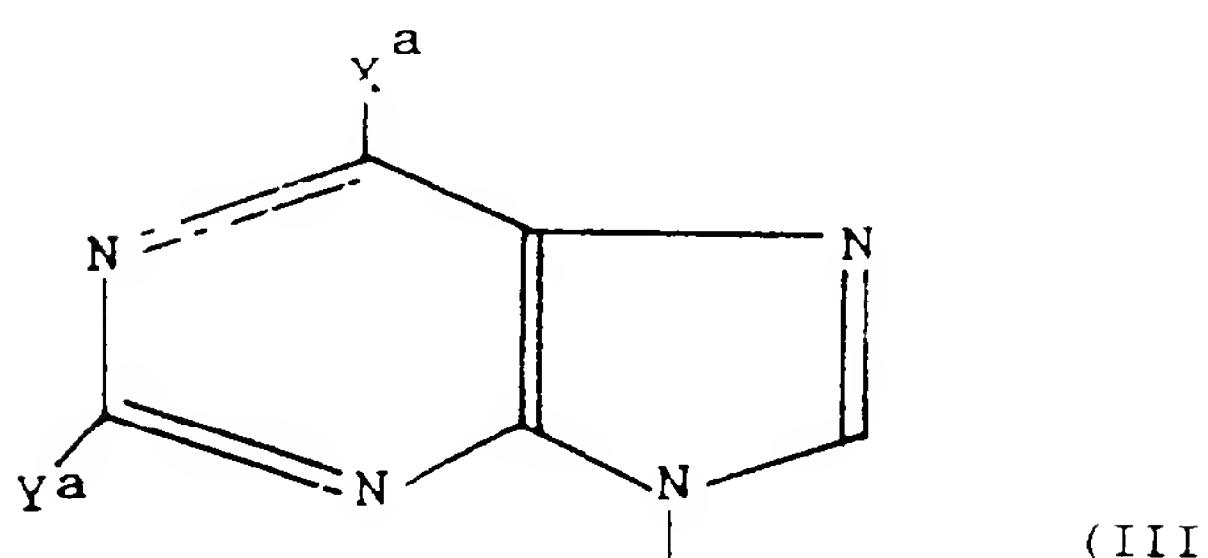
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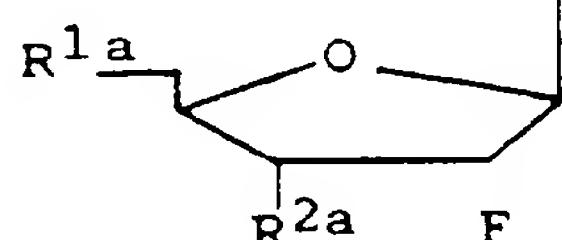


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(wherein X^a, Y^a, R^{1a} and R^{2a} respectively represent the groups X, Y, R¹ and R² as defined above or a precursor, e.g. protected, form of such a group providing at least one of X, Y, R¹ and R² represents a precursor form) with an agent serving to convert the said precursor form of the group(s) to the desired group(s); and thereafter or simultaneously therewith effecting one or more of the following optional conversions:

(i) reacting a compound of formula (I) wherein at least one of R¹ and R² is a hydroxy group with an appropriate agent serving to convert the said hydroxy group to an alternative group represented by R¹ and/or R².

(ii) reacting a compound of formula (I) wherein R¹ and/or R² are not hydroxy with an agent serving to convert the said R¹ and/or R² group to a hydroxy group.

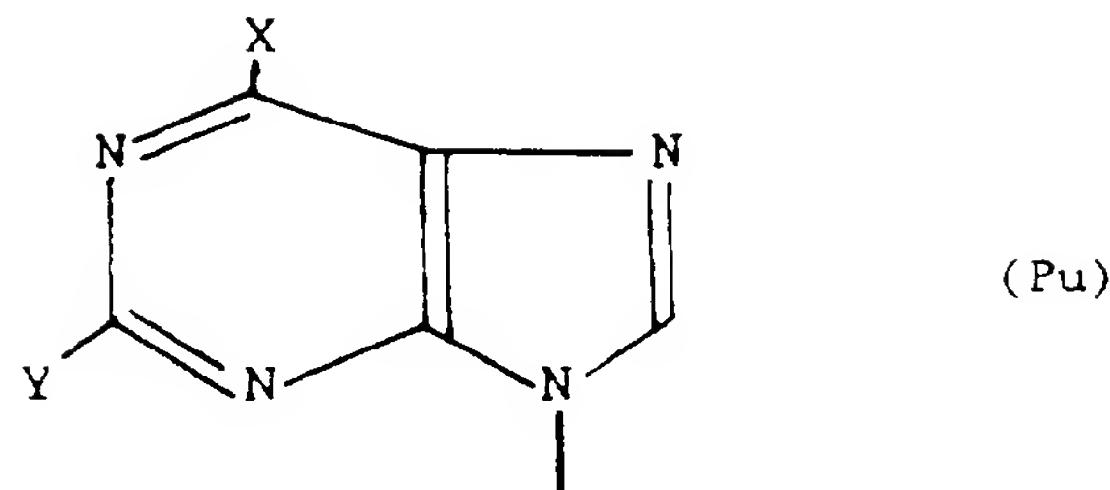
and bringing the resulting compound of formula (I) together with one or more pharmaceutically acceptable carriers to form the said pharmaceutical formulation.

(wherein Y and X are as defined above) or a pharmaceutically acceptable salt thereof which comprises preparing said compound by a process which comprises either:

(A) reacting a purine base of formula Pu wherein Pu represents the purine residue:-

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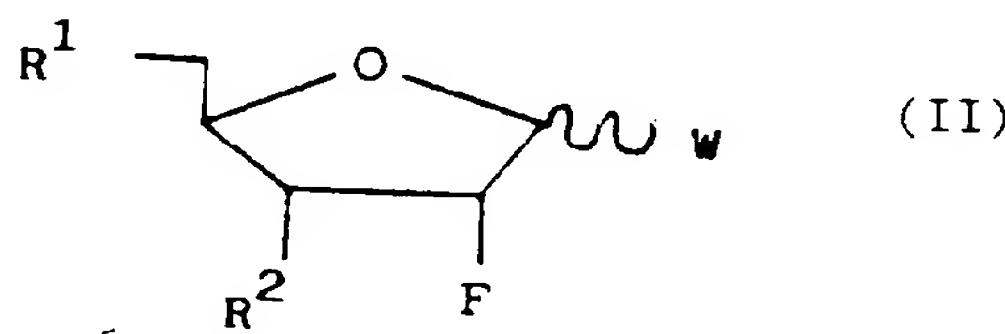
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(wherein X and Y are as described above), or a salt thereof, with a compound of formula (II)

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20 wherein R¹ and R² are as hereinbefore defined, and W is either a phosphate ester or a salt thereof or a purine or pyrimidine moiety (other than Pu) to form a compound of formula (I); (B) reacting a compound of formula (III)

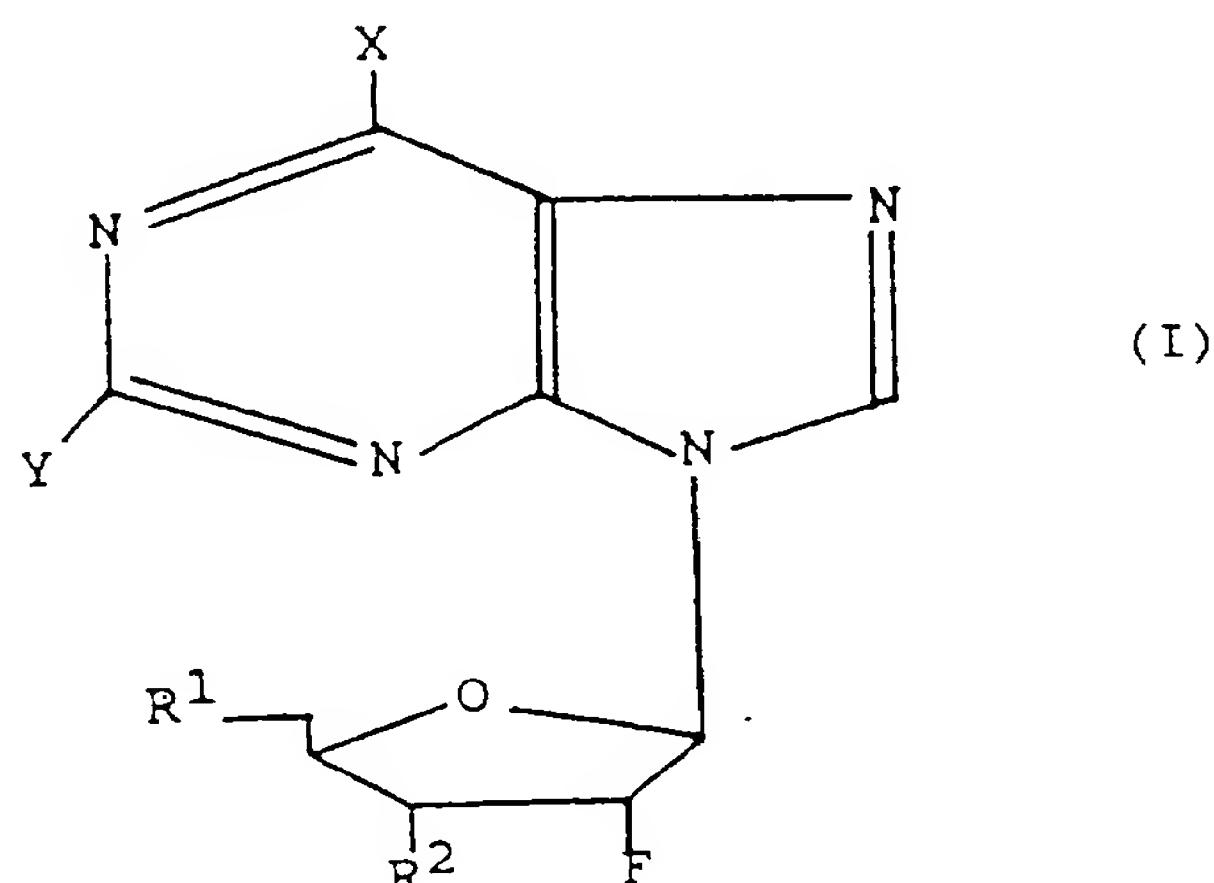
Patentansprüche

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Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE

1. Verbindung der Formel (I)

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worin bedeuten:

Y H oder NH₂;

50 X eine Gruppe -NR³R⁴, worin R³ und R⁴, welche gleich oder verschieden sein können, jeweils Wasserstoff, C₁₋₆-Alkyl, C₂₋₆-Alkenyl, C₃₋₇-Cycloalkyl, wobei jede Gruppe wahlweise durch ein oder mehrere Halogene substituiert ist, bedeuten, oder X ist eine Gruppe -Z-R⁵, worin Z Sauerstoff oder Schwefel ist und R⁵ die gleiche Definition wie R³ besitzt, oder X ist Halogen oder Wasserstoff; und

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R¹ und R², welche gleich oder verschieden sein können, jeweils:

- eine Hydroxygruppe;
- eine Gruppe -OCOR⁶H, worin R⁶ eine zweiwertige Gruppe ist, welche ein gerad- oder verzweigtkettiges C₁₋₆-Alkylen, C₂₋₆-Alkenylen oder C₃₋₇-Cycloalkylen ist, das jeweils wahlweise durch eine oder mehrere

Hydroxygruppen substituiert ist;

- eine Gruppe $-\text{OCO}_2\text{R}^7\text{H}$, worin R^7 eine kovalente Bindung oder wie für R^6 definiert ist;
- eine Gruppe $-\text{OCOR}^6-\text{COOR}^8$, worin R^6 wie oben definiert ist und R^8 aus Wasserstoff oder gerad- oder verzweigtkettigem C_{1-6} -Alkyl oder C_{1-6} -Alkenyl, das jeweils wahlweise durch eine oder mehrere Hydroxygruppen substituiert ist, gewählt ist;
- eine Gruppe $-\text{OCOR}^7-\text{Z-Ar}$, worin R^7 wie oben definiert ist, Z entweder eine kovalente Bindung oder ein Sauerstoff ist, und Ar ein unsubstituierter oder durch ein oder mehrere Halogen, C_{1-6} -Alkyl oder C_{1-6} -Alkoxy substituierter aromatischer Ring ist;
- eine Gruppe $-\text{OR}^6\text{H}$, worin R^6 wie oben definiert ist;
- eine Gruppe $-\text{OR}^6-\text{Z-Ar}$, worin R^6 , Z und Ar wie oben definiert sind;
- eine Gruppe $-\text{OCOCHR}^9\text{NR}^{10}\text{R}^{11}$, worin R^{10} und R^{11} wie für R^8 definiert sind und R^9
 - Wasserstoff,
 - gerad- oder verzweigtkettiges C_{1-4} -Alkyl, das wahlweise durch eine oder mehrere Hydroxy-, Mercapto-, C_{1-3} -Alkoxy- oder C_{1-3} -Alkylthiogruppen substituiert ist,
 - eine Gruppe $\text{R}^{12}-\text{A}$ ist, worin R^{12} eine C_{1-4} -Alkylengruppe ist, die wahlweise durch eine oder mehrere Hydroxygruppen substituiert ist, und A eine Gruppe $-\text{COOH}$, $-\text{CONH}_2$, $-\text{NH}_2$ oder $-\text{NH-C(NH)NH}_2$ ist oder A ist ein 4- bis 11-gliedriges aromatisches oder nichtaromatisches, cyclisches oder heterocyclisches Ringsystem, enthaltend 3 bis 10 Kohlenstoffatome und 0, 1, 2 oder 3 Ring-Stickstoffatome, wobei die Ring-Kohlenstoffatome und/oder -Stickstoffatome wahlweise durch eine oder mehrere Hydroxygruppen substituiert sind;
- eine Gruppe $-\text{OCO-R}^{13}$, worin R^{13} ein 4- bis 7-gliedriger heterocyclischer Ring ist, enthaltend 3 bis 6 Kohlenstoffatome und 0, 1 oder 2 Stickstoffatome, wobei die Ring-Kohlenstoffatome und/oder -Stickstoffatome wahlweise durch eine oder mehrere Hydroxygruppen substituiert sind; oder
- eine Mono-, Di- oder Tri-Phosphatestergruppe oder ein pharmazeutisch annehmbares Salz davon;

verschieden von Verbindungen der Formel (I), worin R^1 und R^2 jeweils Hydroxy sind oder R^1 ein Mono-, Di- oder Tri-Phosphat-5'-ester ist und R^2 Hydroxy ist und entweder (a) X OH ist und Y NH_2 oder H ist; oder (b) X NH_2 ist und Y H ist.

2. Verbindung nach Anspruch 1, **dadurch gekennzeichnet**, daß R^1 und R^2 entweder beide OH sind oder R^1 ein Mono-Phosphat ist und R^2 OH ist, Y H oder NH_2 ist und X eine Gruppe $-\text{NR}^3\text{R}^4$ ist, worin R^3 und R^4 , die gleich oder verschieden sein können, H oder C_{1-6} -Alkyl bedeuten, mit der Maßgabe, daß R^3 und R^4 nicht beide Wasserstoff sind, oder X eine Gruppe Z-R^5 ist, worin Z O oder S ist und R^5 C_{1-6} -Alkyl ist, oder X Halogen oder Wasserstoff ist, oder ein pharmazeutisch annehmbares Salz hiervon.
3. Verbindung nach Anspruch 1 oder 2, **dadurch gekennzeichnet**, daß R^1 und R^2 beide OH sind, Y NH_2 ist und X H , NH_2 oder Z-R^5 ist, worin Z O ist und R^5 C_{1-6} -Alkyl ist, oder ein pharmazeutisch annehmbares Salz hiervon.
4. 2,6-Diamino-9-(2-desoxy-2-fluor- β -D-ribofuranosyl)-9H-purin und pharmazeutisch annehmbare Salze hiervon.
5. 2-Diamino-9-(2-desoxy-2-fluor- β -D-ribofuranosyl)-9H-purin und pharmazeutisch annehmbare Salze hiervon.
6. 2-Amino-9-(2-desoxy-2-fluor- β -D-ribofuranosyl)-6-methoxy-9H-purin und pharmazeutisch annehmbare Salze hiervon.
7. Verbindung nach mindestens einem der Ansprüche 1 bis 6 zur Verwendung in der medizinischen Therapie.
8. Verwendung einer Verbindung, wie in mindestens einem der Ansprüche 1 bis 6 definiert, bei der Herstellung eines Arzneimittels zur Behandlung oder Prophylaxe einer Infektion.
9. Verwendung einer Verbindung nach Anspruch 8, wobei die Infektion eine Virusinfektion ist.
10. Verwendung einer Verbindung nach Anspruch 8, wobei die Infektion eine Protozoeninfektion ist.
11. Verwendung einer Verbindung nach Anspruch 8, wobei die Virusinfektion eine Influenzavirusinfektion ist.

12. Verwendung einer Verbindung nach Anspruch 8, wobei die Virusinfektion eine respiratorische Synzytiumvirusinfektion ist.

13. Verwendung einer Verbindung nach Anspruch 8, wobei die Protozoeninfektion eine *Trichomonas vaginalis*-Infektion ist.

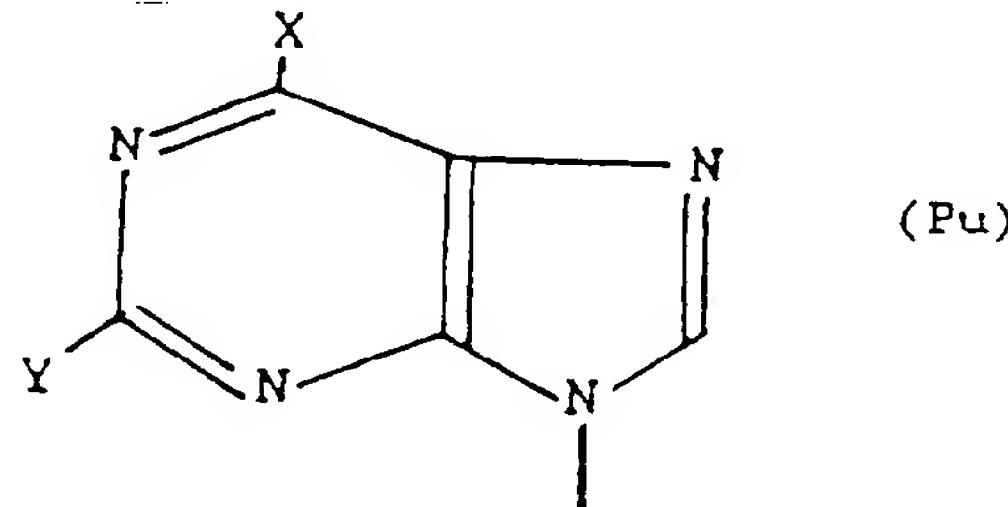
14. Verwendung einer Verbindung nach Anspruch 8, wobei die Protozoeninfektion eine *Giardia lamblia*-Infektion ist.

15. Pharmazeutische Zubereitung, umfassend eine Verbindung nach mindestens einem der Ansprüche 2 bis 7, zusammen mit einem pharmazeutisch annehmbaren Träger hierfür.

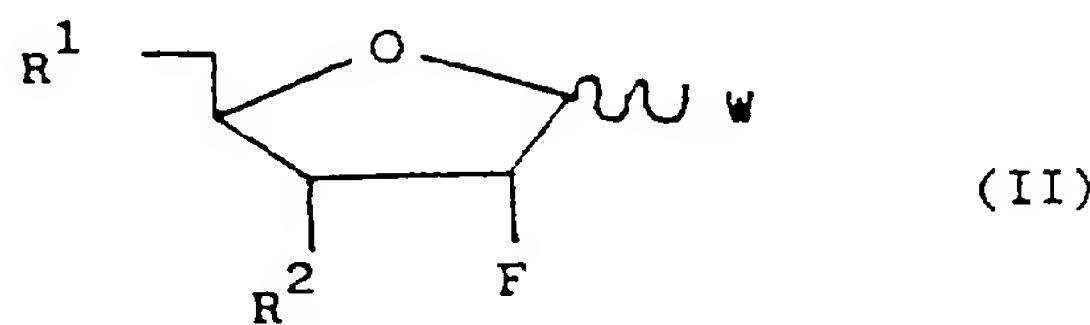
16. Pharmazeutische Zubereitung nach Anspruch 15, welche zur oralen oder nasalen Verabreichung adaptiert ist.

17. Verfahren zur Herstellung einer Verbindung der Formel (I) gemäß den Ansprüchen 1 bis 6 oder eines pharmazeutisch annehmbaren Salzes hiervon, umfassend:

(A) Umsetzen einer Purin-Base der Formel Pu, wobei PuH den Purinrest bedeutet:

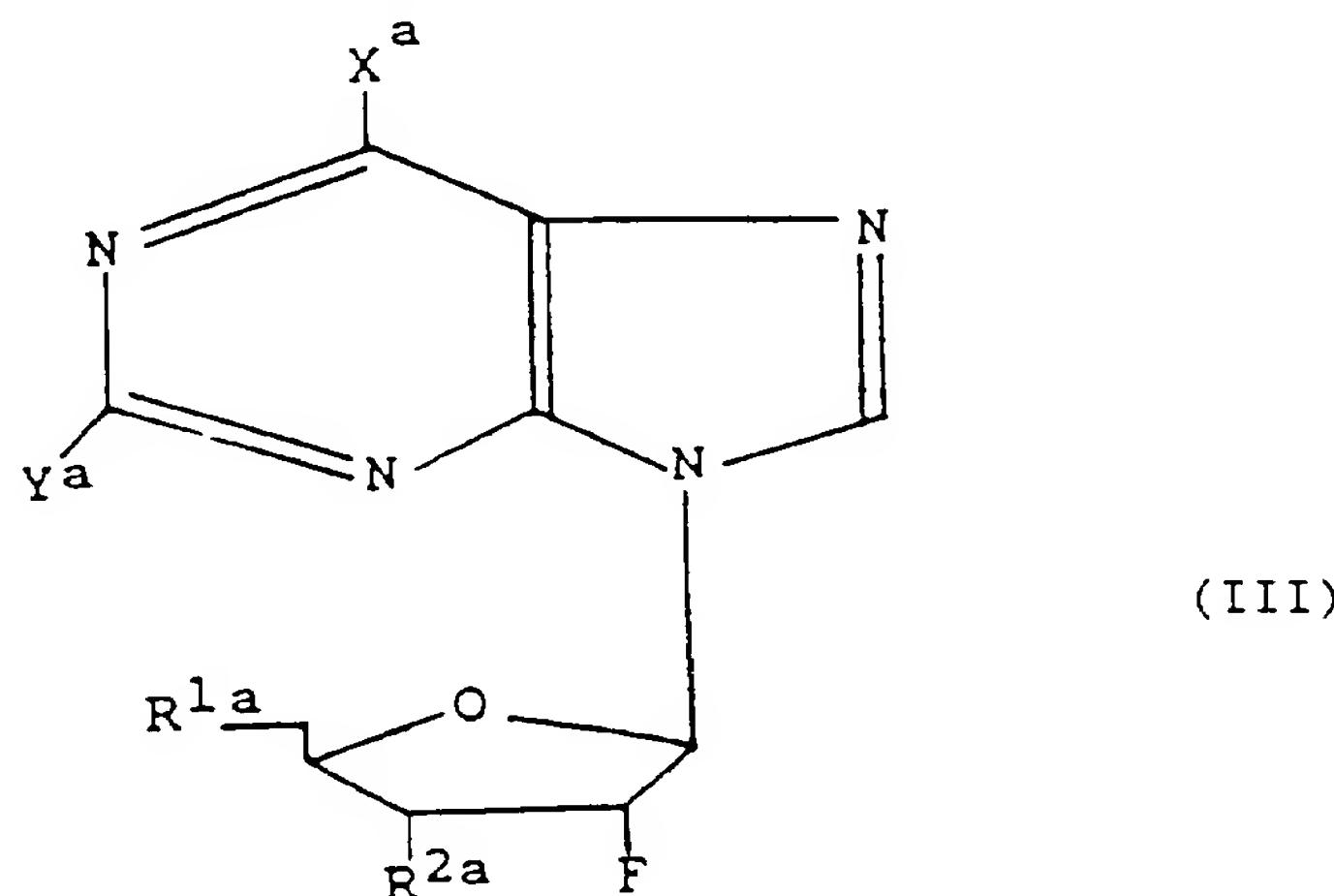


(worin X und Y wie oben beschrieben sind) oder eines Salzes hiervon, mit einer Verbindung der Formel (II)



worin R¹ und R² wie oben definiert sind und W entweder ein Phosphatester oder ein Salz hiervon oder eine Purin- oder Pyrimidingruppe (von Pu verschiedenen) ist, zur Bildung einer Verbindung der Formel (I);

(B) Umsetzen einer Verbindung der Formel (III)



(worin X^a, Y^a, R^{1a} und R^{2a} jeweils die Gruppen X, Y, R¹ und R², wie oben definiert, oder einen Vorläufer hiervon, bedeuten, beispielsweise die geschützte Form einer solchen Gruppe, vorausgesetzt mindestens eines von X, Y, R¹ und R² bedeutet eine Vorläuferform) mit einem Mittel, das dazu dient, die Vorläuferform der Gruppe(n)

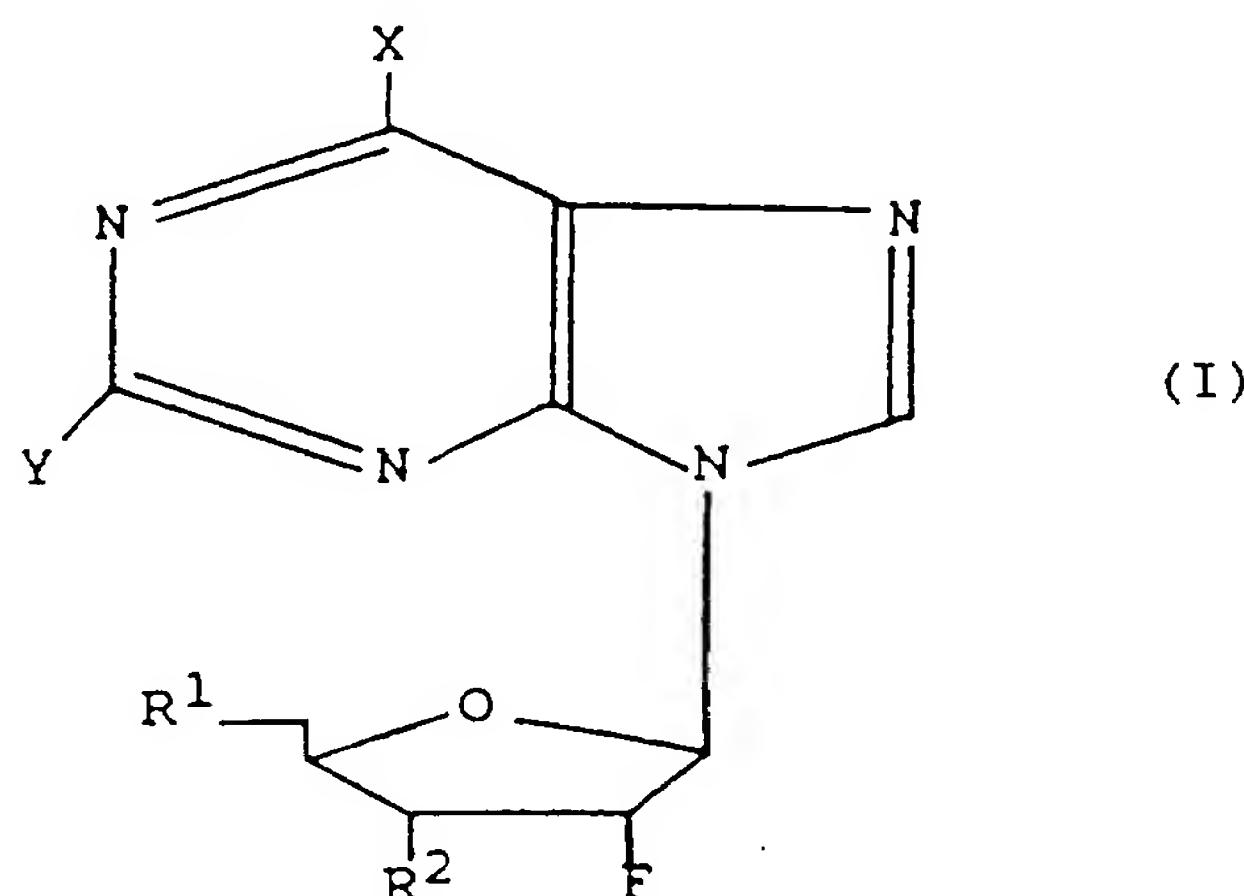
in die erwünschte Gruppe(n) umzuwandeln;
und danach oder gleichzeitig hiermit Bewirken einer oder mehrerer der folgenden wahlweisen Umwandlungen:

5 (i) Umsetzen einer Verbindung der Formel (I), worin mindestens eines von R¹ und R² eine Hydroxygruppe ist, mit einem geeigneten Mittel, das dazu dient, diese Hydroxygruppe in eine durch R¹ und/oder R² angegebene, alternative Gruppe umzuwandeln;

10 (ii) Umsetzen einer Verbindung der Formel (I), worin R¹ und/oder R² nicht Hydroxy sind, mit einem Mittel, das dazu dient, die R¹- und/oder R²-Gruppe in eine Hydroxygruppe umzuwandeln.

Patentansprüche für folgende Vertragsstaaten : ES, GR

15 1. Verfahren zur Herstellung einer Verbindung der Formel (I)



worin bedeuten:

35 Y H oder NH₂;

X eine Gruppe -NR³R⁴, worin R³ und R⁴, welche gleich oder verschieden sein können, jeweils Wasserstoff, C₁₋₆-Alkyl, C₂₋₆-Alkenyl, C₃₋₇-Cycloalkyl, wobei jede Gruppe wahlweise durch ein oder mehrere Halogene substituiert ist, bedeuten, oder X ist eine Gruppe -Z-R⁵, worin Z Sauerstoff oder Schwefel ist und R⁵ die gleiche Definition wie R³ besitzt, oder X ist Halogen oder Wasserstoff; und

40 R¹ und R², welche gleich oder verschieden sein können, jeweils:

- 45 - eine Hydroxygruppe;
- eine Gruppe -OCOR⁶H, worin R⁶ eine zweiwertige Gruppe ist, welche ein gerad- oder verzweigtkettiges C₁₋₆-Alkylen, C₂₋₆-Alkenylen oder C₃₋₇-Cycloalkylen ist, das jeweils wahlweise durch eine oder mehrere Hydroxygruppen substituiert ist;
- eine Gruppe -OCO₂R⁷H, worin R⁷ eine kovalente Bindung oder wie für R⁶ definiert ist;
- eine Gruppe -OCOR⁶-COOR⁸, worin R⁶ wie oben definiert ist und R⁸ aus Wasserstoff oder gerad- oder verzweigtkettigem C₁₋₆-Alkyl oder C₁₋₆-Alkenyl, das jeweils wahlweise durch eine oder mehrere Hydroxygruppen substituiert ist, gewählt ist;
- eine Gruppe -OCOR⁷-Z-Ar, worin R⁷ wie oben definiert ist, Z entweder eine kovalente Bindung oder ein Sauerstoff ist, und Ar ein unsubstituierter oder durch ein oder mehrere Halogen, C₁₋₆-Alkyl oder C₁₋₆-Alkoxy substituierter aromatischer Ring ist;
- eine Gruppe -OR⁶H, worin R⁶ wie oben definiert ist;
- eine Gruppe -OR⁶-Z-Ar, worin R⁶, Z und Ar wie oben definiert sind;
- eine Gruppe -OCOCHR⁹NR¹⁰R¹¹, worin R¹⁰ und R¹¹ wie für R⁸ definiert sind und R⁹

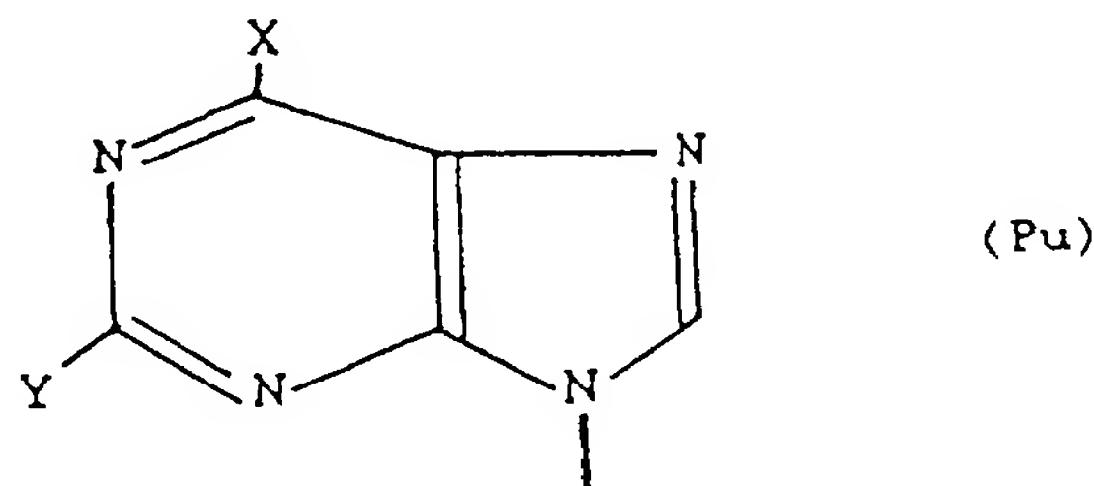
-- Wasserstoff,
 -- gerad- oder verzweigtketiges C₁₋₄-Alkyl, das wahlweise durch eine oder mehrere Hydroxy-, Mercapto-, C₁₋₃-Alkoxy- oder C₁₋₃-Alkylthiogruppen substituiert ist,
 -- eine Gruppe R¹²-A ist, worin R¹² eine C₁₋₄-Alkylengruppe ist, die wahlweise durch eine oder mehrere Hydroxygruppen substituiert ist, und A eine Gruppe -COOH, -CONH₂, -NH₂ oder -NH-C(NH)NH₂ ist oder A ist ein 4- bis 11-gliedriges aromatisches oder nichtaromatisches, cyclisches oder heterocyclisches Ringsystem, enthaltend 3 bis 10 Kohlenstoffatome und 0, 1, 2 oder 3 Ring-Stickstoffatome, wobei die Ring-Kohlenstoffatome und/oder -Stickstoffatome wahlweise durch eine oder mehrere Hydroxygruppen substituiert sind;

10 - eine Gruppe -OCO-R¹³, worin R¹³ ein 4- bis 7-gliedriger heterocyclischer Ring ist, enthaltend 3 bis 6 Kohlenstoffatome und 0, 1 oder 2 Stickstoffatome, wobei die Ring-Kohlenstoffatome und/oder -Stickstoffatome wahlweise durch eine oder mehrere Hydroxygruppen substituiert sind; oder

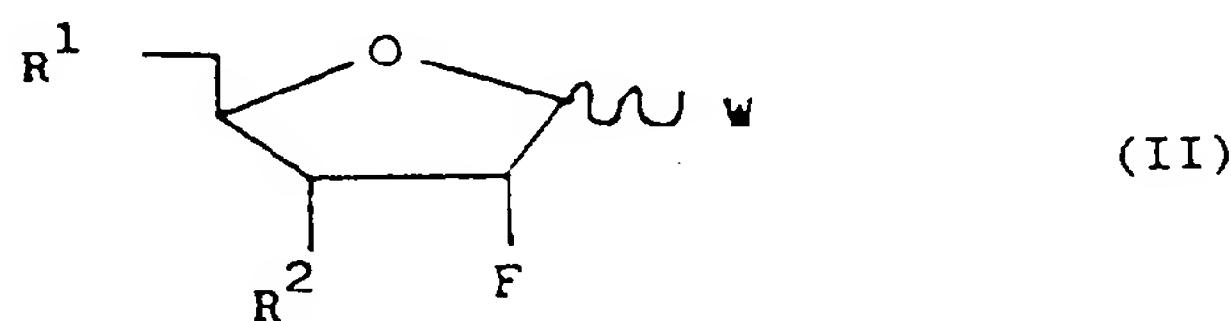
15 - eine Mono-, Di- oder Tri-Phosphategruppe oder ein pharmazeutisch annehmbares Salz davon;

verschieden von Verbindungen der Formel (I), worin R¹ und R² jeweils Hydroxy sind oder R¹ ein Mono-, Di- oder Tri-Phosphat-5'-ester ist und R² Hydroxy ist und entweder (a) X OH ist und Y NH₂ oder H ist; oder (b) X NH₂ ist und Y H ist, umfassend entweder

20 (A) Umsetzen einer Purin-Base der Formel PuH, wobei Pu den Purinrest bedeutet:

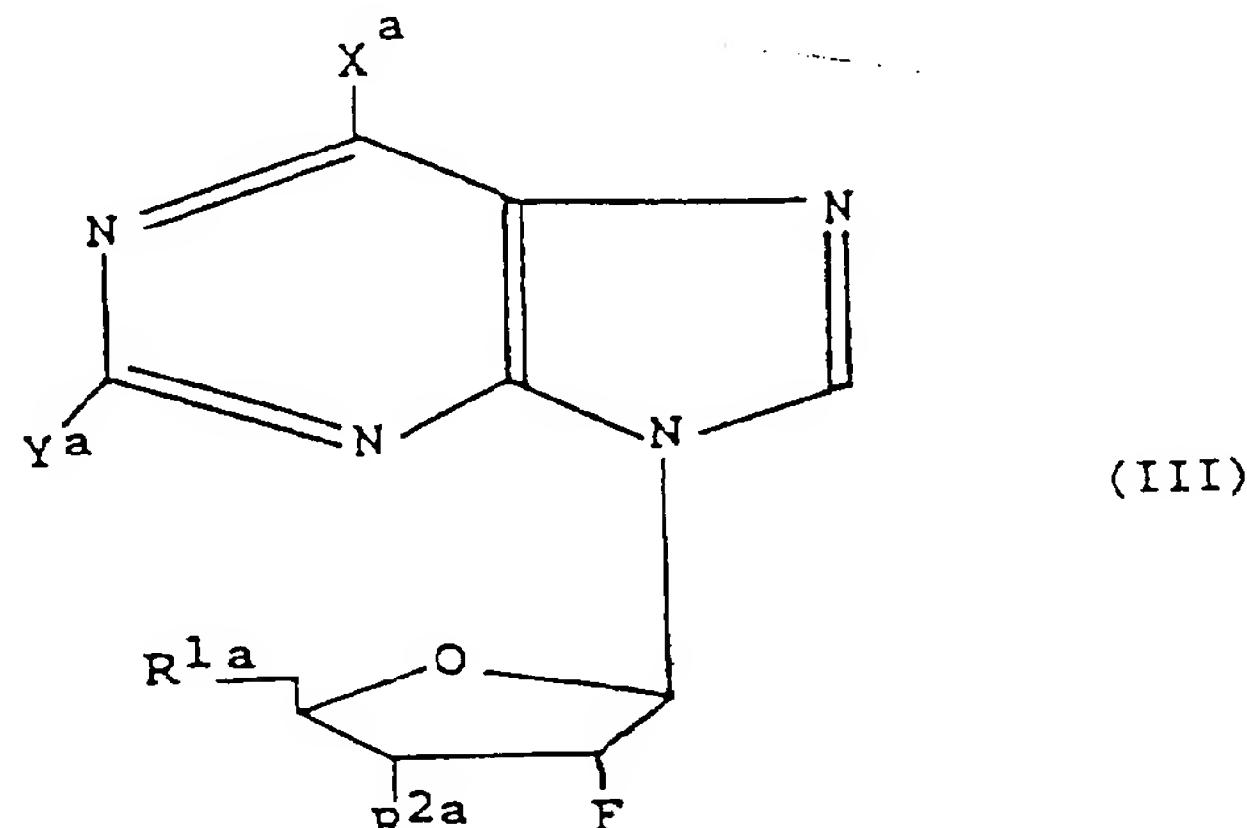


30 (worin X und Y wie oben beschrieben sind) oder eines Salzes hiervon, mit einer Verbindung der Formel (II)



40 worin R¹ und R² wie oben definiert sind und W entweder ein Phosphatester oder ein Salz hiervon oder eine Purin- oder Pyrimidingruppe (von Pu verschiedenen) ist, zur Bildung einer Verbindung der Formel (I);

(B) Umsetzen einer Verbindung der Formel (III)



55 (worin X^a, Y^a, R^{1a} und R^{2a} jeweils die Gruppen X, Y, R¹ und R², wie oben definiert, oder einen Vorläufer hiervon,

bedeuten, beispielsweise die geschützte Form einer solchen Gruppe, vorausgesetzt mindestens eines von X, Y, R¹ und R² bedeutet eine Vorläuferform) mit einem Mittel, das dazu dient, die Vorläuferform der Gruppe(n) in die erwünschte Gruppe(n) umzuwandeln;

5 und danach oder gleichzeitig hiermit Bewirken einer oder mehrerer der folgenden wahlweisen Umwandlungen:

(i) Umsetzen einer Verbindung der Formel (I), worin mindestens eines von R¹ und R² eine Hydroxygruppe ist, mit einem geeigneten Mittel, das dazu dient, diese Hydroxygruppe in eine durch R¹ und/oder R² angegebene, 10 alternative Gruppe umzuwandeln;

(ii) Umsetzen einer Verbindung der Formel (I), worin R¹ und/oder R² nicht Hydroxy sind, mit einem Mittel, das dazu dient, die R¹- und/oder R²-Gruppe in eine Hydroxygruppe umzuwandeln.

15 2. Verfahren nach Anspruch 1 zur Herstellung einer Verbindung der Formel (I), wobei R¹ und R² entweder beide OH sind oder R¹ ein Mono-Phosphat ist und R² OH ist, Y H oder NH₂ ist und X eine Gruppe -NR³R⁴ ist, worin R³ und R⁴, die gleich oder verschieden sein können, H oder C₁₋₆-Alkyl bedeuten, mit der Maßgabe, daß R³ und R⁴ nicht beide Wasserstoff sind, oder X eine Gruppe Z-R⁵ ist, worin Z O oder S ist und R⁵ C₁₋₆-Alkyl ist, oder X Halogen oder Wasserstoff ist, oder eines pharmazeutisch annehmbaren Salzes hiervon.

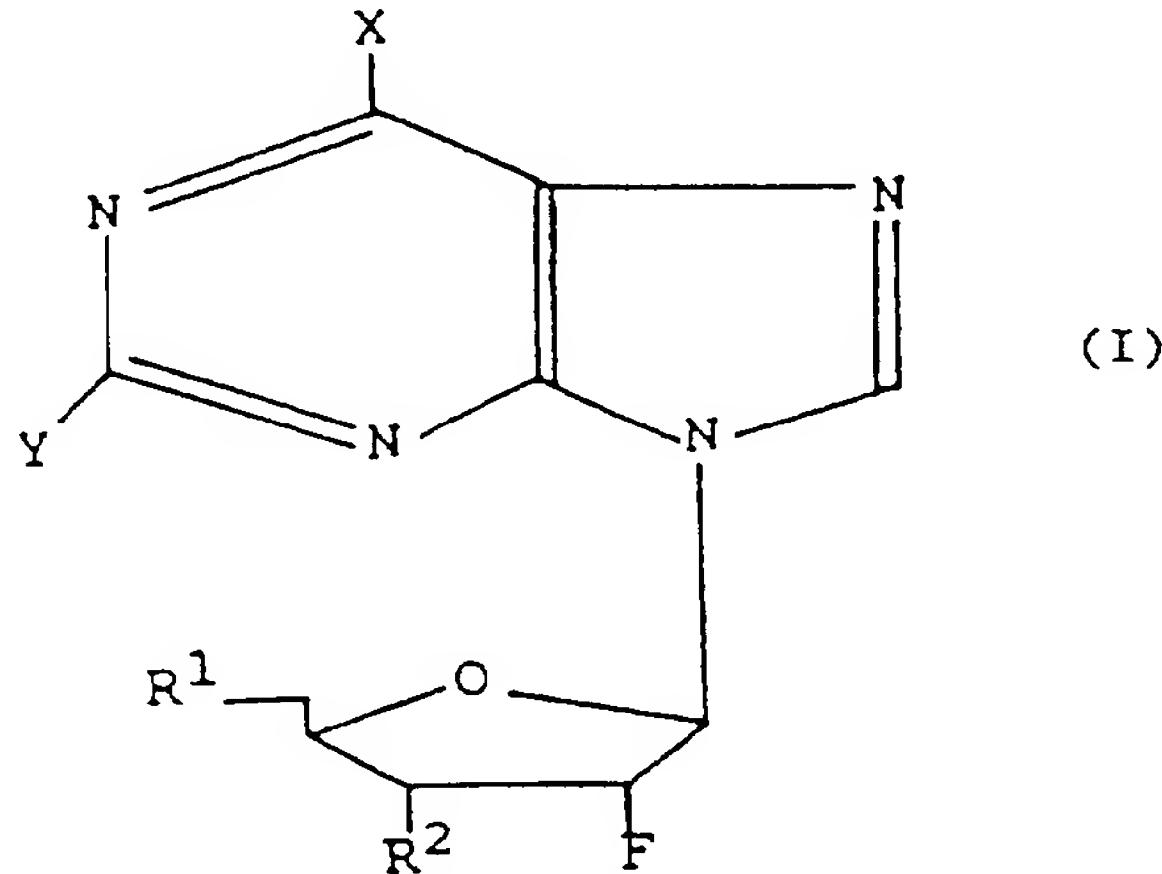
20 3. Verfahren nach Anspruch 1 oder 2 zur Herstellung einer Verbindung der Formel (I), worin R¹ und R² beide OH sind, Y NH₂ ist und X H, NH₂ oder Z-R⁵ ist, worin Z O ist und R⁵ C₁₋₆-Alkyl ist, oder eines pharmazeutisch annehmbaren Salzes hiervon.

25 4. Verfahren zur Herstellung der Verbindung 2,6-Diamino-9-(2-desoxyfluor-β-D-ribofuranosyl)-9H-purin und pharmazeutisch annehmbarer Salze hiervon.

5. Verfahren zur Herstellung der Verbindung 2-Amino-9-(2-desoxy-2-fluor-β-D-ribofuranosyl)-9H-purin und pharmazeutisch annehmbarer Salze hiervon.

30 6. Verfahren zur Herstellung der Verbindung 2-Amino-9-(2-desoxy-2-fluor-β-D-ribofuranosyl)-6-methoxy-9H-purin und pharmazeutisch annehmbarer Salze hiervon.

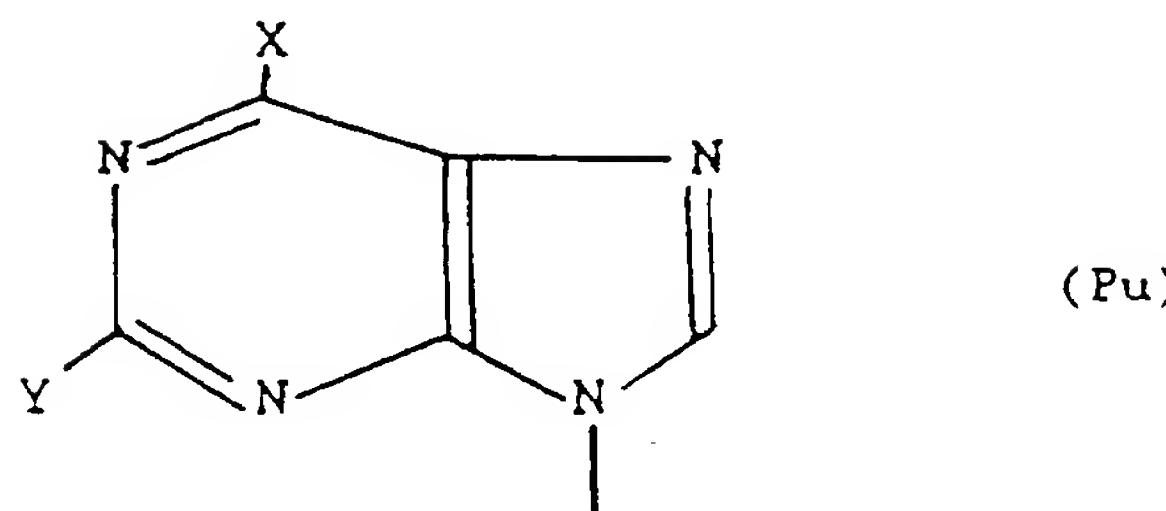
7. Verfahren zur Herstellung einer pharmazeutischen Zubereitung, enthaltend eine Verbindung der Formel (I)



(worin Y und X wie oben definiert sind) oder eines pharmazeutisch annehmbaren Salzes hiervon, umfassend das Herstellen der Verbindung durch ein Verfahren, umfassend entweder:

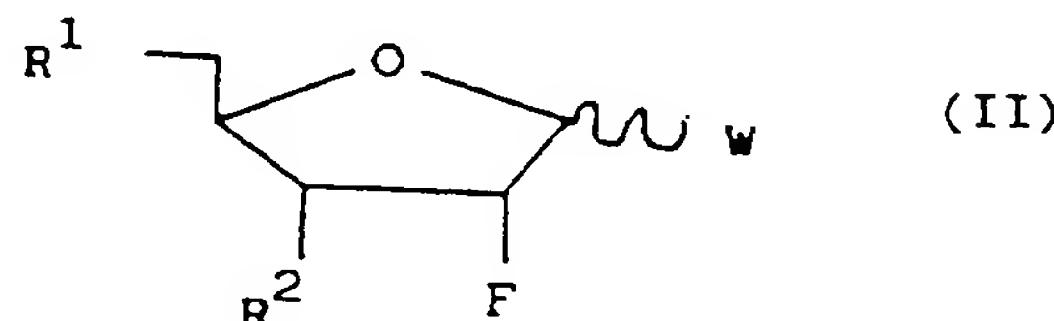
(A) Umsetzen einer Purinbase der Formel PuH, worin Pu den Purinrest bedeutet:

5



10 (worin X und Y wie oben beschrieben sind) oder eines Salzes hiervon, mit einer Verbindung der Formel (II)

15

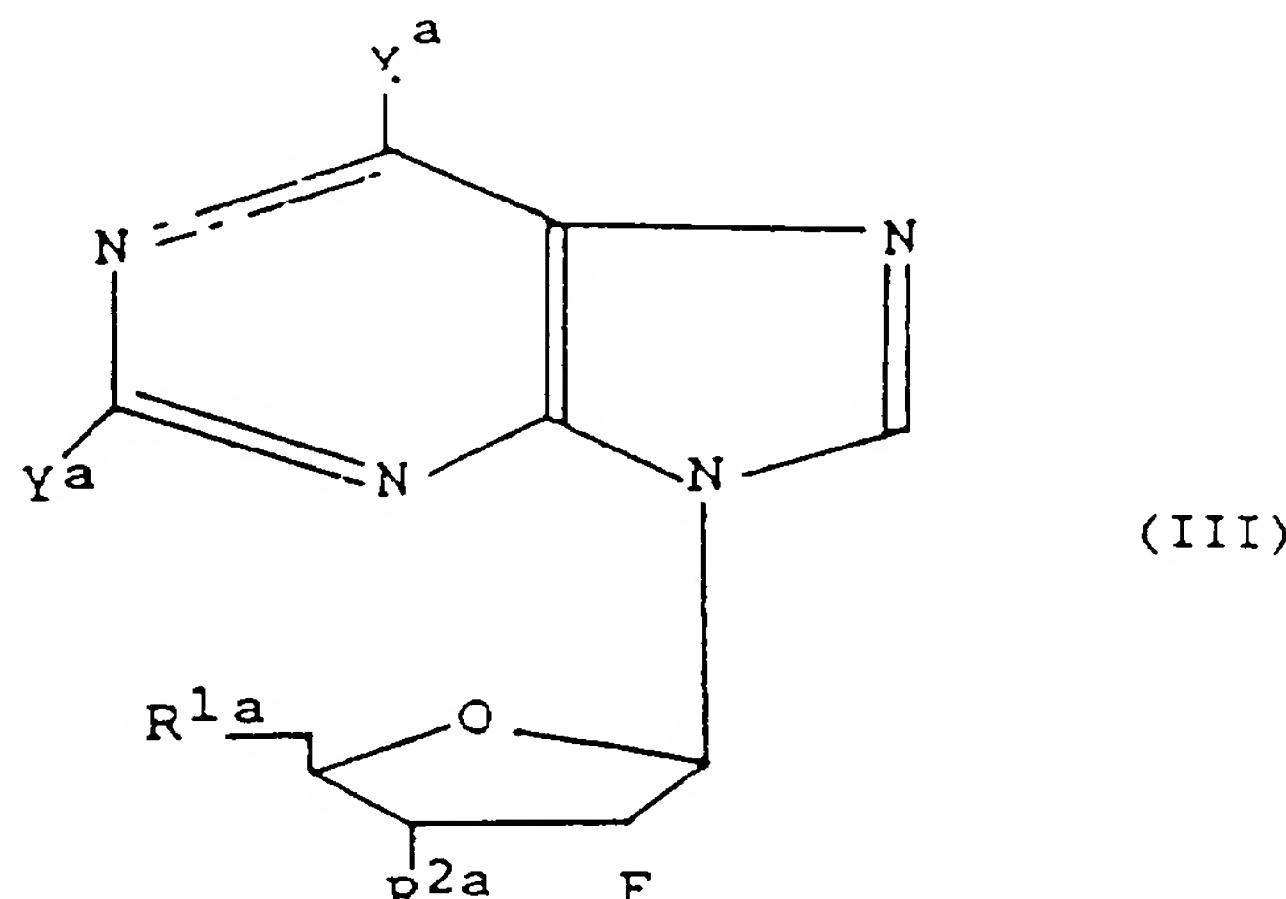


20 worin R¹ und R² wie oben definiert sind und W entweder ein Phosphatester oder ein Salz hiervon oder eine Purin- oder Pyrimidingruppe (von Pu verschieden) ist, zur Bildung einer Verbindung der Formel (I);

25

(B) Umsetzen einer Verbindung der Formel (III)

30



35

40 (worin X^a, Y^a, R^{1a} und R^{2a} jeweils die Gruppen X, Y, R¹ und R², wie oben definiert, oder einen Vorläufer hiervon, bedeuten, beispielsweise die geschützte Form einer solchen Gruppe, vorausgesetzt mindestens eines von X, Y, R¹ und R² bedeutet eine Vorläuferform) mit einem Mittel, das dazu dient, die vorläuferform der Gruppe(n) in die erwünschte Gruppe(n) umzuwandeln;

45 und danach oder gleichzeitig hiermit Bewirken einer oder mehrerer der folgenden wahlweisen Umwandlungen:

45

(i) Umsetzen einer Verbindung der Formel (I), worin mindestens eines von R¹ und R² eine Hydroxygruppe ist, mit einem geeigneten Mittel, das dazu dient, diese Hydroxygruppe in eine durch R¹ und/oder R² angegebene, alternative Gruppe umzuwandeln;

50

(ii) Umsetzen einer Verbindung der Formel (I), worin R¹ und/oder R² nicht Hydroxy sind, mit einem Mittel, das dazu dient, die R¹- und/oder R²-Gruppe in eine Hydroxygruppe umzuwandeln,

55 und Zusammenbringen der resultierenden Verbindung der Formel (I) mit einem oder mehreren pharmazeutisch annehmbaren Trägern zur Bildung der pharmazeutischen Zubereitung.

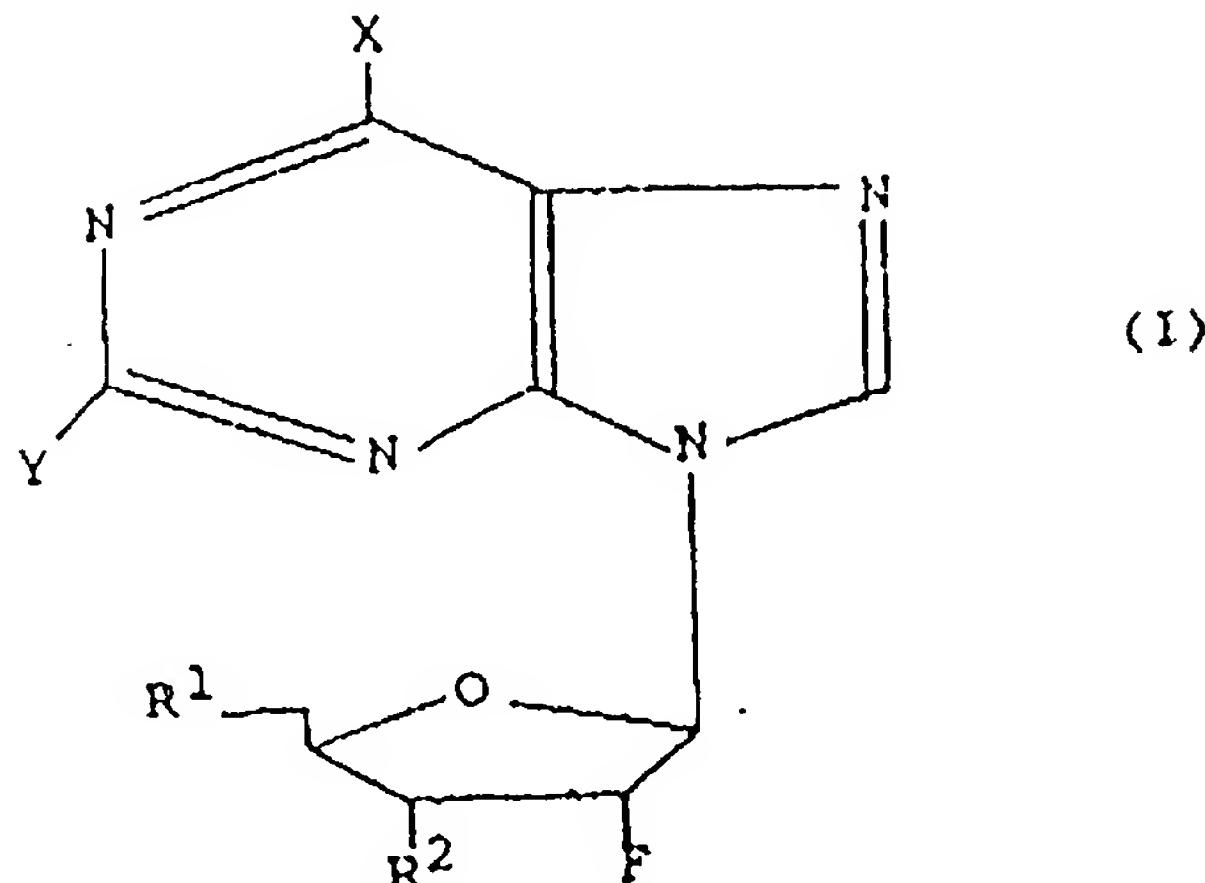
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Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE

5

1. Composé de formule (I)



10

dans laquelle Y est H ou NH₂ ;

25

X est un groupe -NR³R⁴ dans lequel R³ et R⁴, qui peuvent être identiques ou différents, représentent chacun un atome d'hydrogène ;

30

un groupe alkyle en C₁₋₆, alkényle en C₂₋₆, cycloalkyle en C₃₋₇, chaque groupe étant éventuellement substitué par un ou plusieurs atomes d'halogène, ou X est un groupe Z-R⁵ dans lequel Z est un atome d'oxygène ou de soufre et R⁵ a la même définition que R³, ou X est un atome d'halogène ou d'hydrogène ; et

35

R¹ et R², qui peuvent être identiques ou différents, représentent chacun :

40

- un groupe hydroxy ;
- un groupe -OCOR⁶H où R⁶ est un groupe bivalent qui est une chaîne linéaire ou ramifiée alkylène en C₁₋₆, alkénylène en C₂₋₆ ou cycloalkylène en C₃₋₇, chacune étant éventuellement substituée par un ou plusieurs groupes hydroxy ;

45

- un groupe -OCO₂R⁷H où R⁷ est une liaison covalente ou est tel que défini pour R⁶ ;
- un groupe -OCOR⁶-COOR⁸ où R⁶ est tel que défini ci-dessus et R⁸ est choisi parmi un atome d'hydrogène, ou une chaîne linéaire ou ramifiée alkyle en C₁₋₆ ou alkényle en C₁₋₆, chacune étant éventuellement substituée par un ou plusieurs groupes hydroxy ;
- un groupe -OCOR⁷-z-Ar où R⁷ est tel que défini ci-dessus, Z est une liaison covalente ou un atome d'oxygène et Ar est un cycle aromatique non substitué ou substitué par un ou plusieurs atomes d'halogène, un groupe alkyle en C₁₋₆ ou alkoxy en C₁₋₆ ;
- un groupe -OR⁶H où R⁶ est tel que défini ci-dessus ;
- un groupe -OR⁶-z-Ar où R⁶, Z et Ar sont tels que définis ci-dessus ;
- un groupe -OCOCHR⁹NR¹⁰R¹¹ où R¹⁰ et R¹¹ sont tels que définis pour R⁸, et R⁹ est
 - un atome d'hydrogène,
 - une chaîne linéaire ou ramifiée alkyle en C₁₋₄ éventuellement substituée par un ou plusieurs groupes hydroxy, mercapto, alkoxy en C₁₋₃ ou alkylthio en C₁₋₃

55

5 -- un groupe R¹²-A où R¹² est un groupe alkylène en C₁₋₄ éventuellement substitué par un ou plusieurs groupes hydroxy et A est un groupe -COOH, -CONH₂, -NH₂ ou -NH-C(NH)NH₂ ou A est un système cyclique ou hétérocyclique aromatique ou non aromatique de 4 à 11 éléments contenant 3 à 10 atomes de carbone et 0, 1, 2 ou 3 atomes d'azote dans le cycle, les atomes de carbone et/ou les atomes d'azote du cycle étant éventuellement substitués par un ou plusieurs groupes hydroxy ;

10 - un groupe -OCO-R¹³ où R¹³ est un hétérocycle de 4 à 7 éléments contenant 3 à 6 atomes de carbone et 0, 1 ou 2 atomes d'azote, les atomes de carbone et/ou les atomes d'azote du cycle étant éventuellement substitués par un ou plusieurs groupes hydroxy ; ou

15 - un groupe ester mono-, di- ou tri-phosphate ou un sel pharmaceutiquement acceptable de celui-ci; à l'exclusion des composés de formule (I) dans lesquels R¹ et R² sont chacun un groupe hydroxy ou R¹ est un ester en 5' mono-, di- ou tri-phosphate et R² est un groupe hydroxy et soit (a) X est OH et Y est NH₂ ou H ; soit (b) X est NH₂ et Y est H.

20 2. Composé selon la revendication 1 caractérisé en ce que R¹ et R² sont soit tous deux OH, soit R¹ est un monophosphate et R² est OH, Y est H ou NH₂ et X est un groupe -NR³R⁴ dans lequel R³ et R⁴, qui peuvent être identiques ou différents, représentent H, ou un groupe alkyle en C₁₋₆, à condition que R³ et R⁴ ne soient pas tous deux un atome d'hydrogène, ou X est un groupe Z-R⁵ dans lequel Z est O ou S et R⁵ est un groupe alkyle en C₁₋₆, ou X est un atome d'halogène ou d'hydrogène, ou un sel pharmaceutiquement acceptable de ceux-ci.

25 3. Composé selon la revendication 1 ou 2 caractérisé en ce que R¹ et R² sont tous deux OH, Y est NH₂ et X est H, NH₂ ou Z-R⁵ où Z est O et R⁵ est un groupe alkyle en C₁₋₆, ou un sel pharmaceutiquement acceptable de celui-ci.

30 4. 2,6-diamino-9-(2-désoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine et sels pharmaceutiquement acceptables de celle-ci.

35 5. 2-amino-9-(2-désoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine et sels pharmaceutiquement acceptables de celle-ci.

40 6. 2-amino-9-(2-désoxy-2-fluoro-β-D-ribofuranosyl)-6-méthoxy-9H-purine et sels pharmaceutiquement acceptables de celle-ci.

45 7. Composé selon l'une quelconque des revendications 1 à 6 pour l'utilisation dans une thérapie médicale.

50 8. Utilisation d'un composé tel que défini dans l'une quelconque des revendications 1 à 6, dans la préparation d'un médicament pour le traitement curatif ou préventif d'une infection.

55 9. Utilisation d'un composé tel que revendiqué à la revendication 8 dans laquelle l'infection est une infection virale.

60 10. Utilisation d'un composé tel que revendiqué à la revendication 8 dans laquelle l'infection est une infection due à un protozoaire.

65 11. Utilisation d'un composé tel que revendiqué à la revendication 8 dans laquelle l'infection est une infection par le virus de la grippe.

70 12. Utilisation d'un composé tel que revendiqué à la revendication 8 dans laquelle l'infection virale est une infection par un virus respiratoire syncytial.

75 13. Utilisation d'un composé tel que revendiqué à la revendication 8 dans laquelle l'infection due à un protozoaire est une infection par Trichomonas vaginalis.

80 14. Utilisation d'un composé tel que revendiqué à la revendication 8 dans laquelle l'infection due à un protozoaire est une infection par Giardia lamblia.

85 15. Formulation pharmaceutique comprenant un composé selon l'une quelconque des revendications 2 à 7, avec un véhicule pharmaceutiquement acceptable pour celle-ci.

90 16. Formulation pharmaceutique telle que revendiquée à la revendication 15 adaptée à une administration orale ou

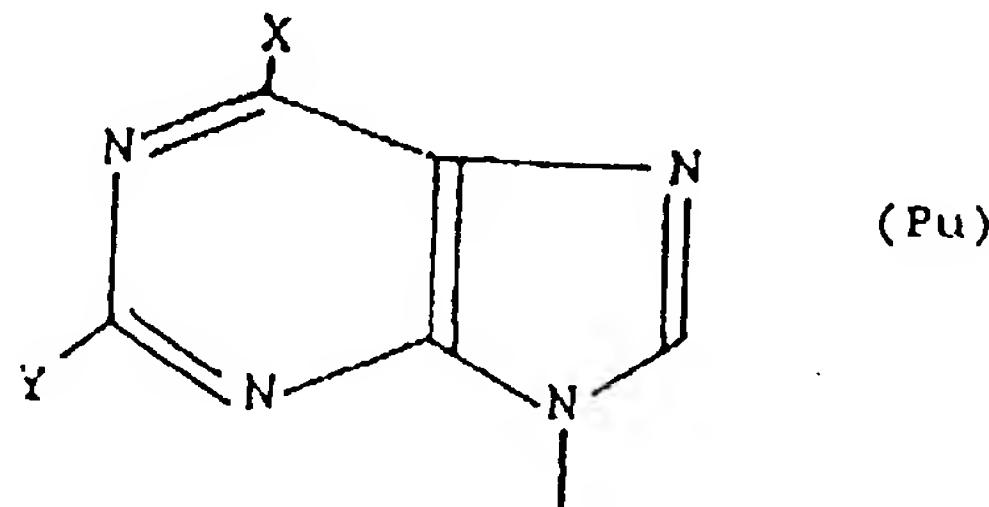
nasale.

17. Procédé pour la préparation d'un composé de formule (I) tel que revendiqué aux revendications 1 à 6 ou un sel pharmaceutiquement acceptable de celui-ci qui comprend les étapes consistant à :

5

(A) faire réagir une base purique de formule Pu dans laquelle PuH représente le résidu purine :

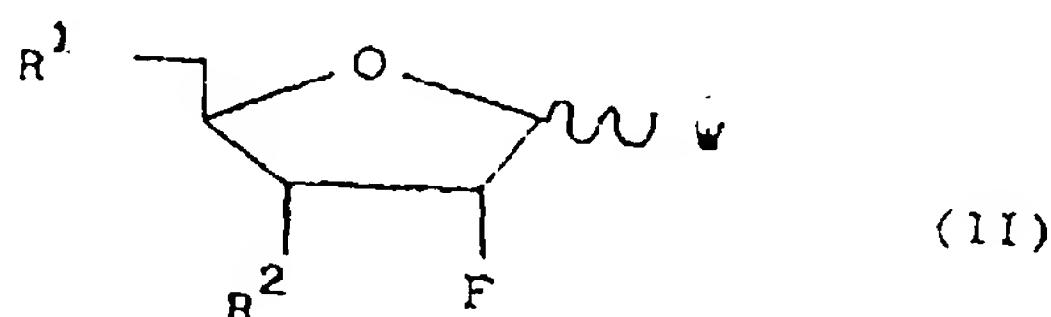
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(où X et Y sont tels que décrits ci-dessus), ou un sel de celui-ci, avec un composé de formule (II)

20

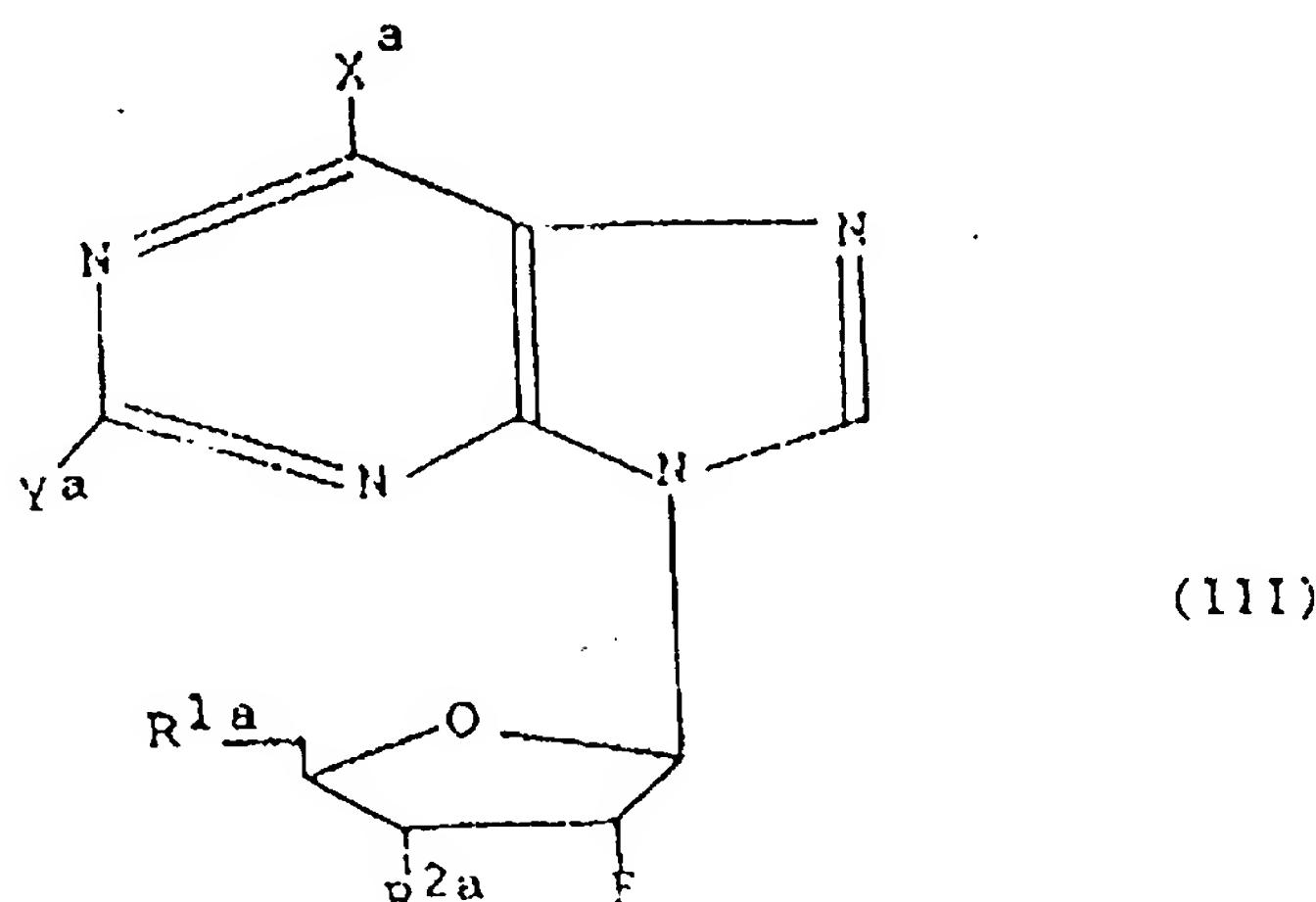


25

dans laquelle R¹ et R² sont tels que définis ci-dessus, et W est soit un ester phosphate ou un sel de celui-ci soit une partie purine ou pyrimidine (autre que Pu) pour former un composé de formule (I) ;

(B) faire réagir un composé de formule (III)

30



40

45

(dans laquelle X^a, Y^a, R^{1a} et R^{2a} représentent respectivement les groupes x, Y, R¹ et R² tels que définis ci-dessus ou une forme précurseur, par exemple protégée, d'un tel groupe fournissant au moins un des groupes X, Y, R¹ et R² représentant une forme précurseur) avec un agent servant à convertir ladite forme précurseur du (des) groupe(s) en le(les) groupe(s) désiré(s) ;

50 et ensuite ou simultanément avec ceci effectuer une ou plusieurs des conversions éventuelles suivantes :

(i) faire réagir un composé de formule (I) dans lequel au moins un des groupes R¹ et R² est un groupe hydroxy avec un agent approprié servant à convertir ledit groupe hydroxy en un autre groupe représenté par R¹ et/ou R².

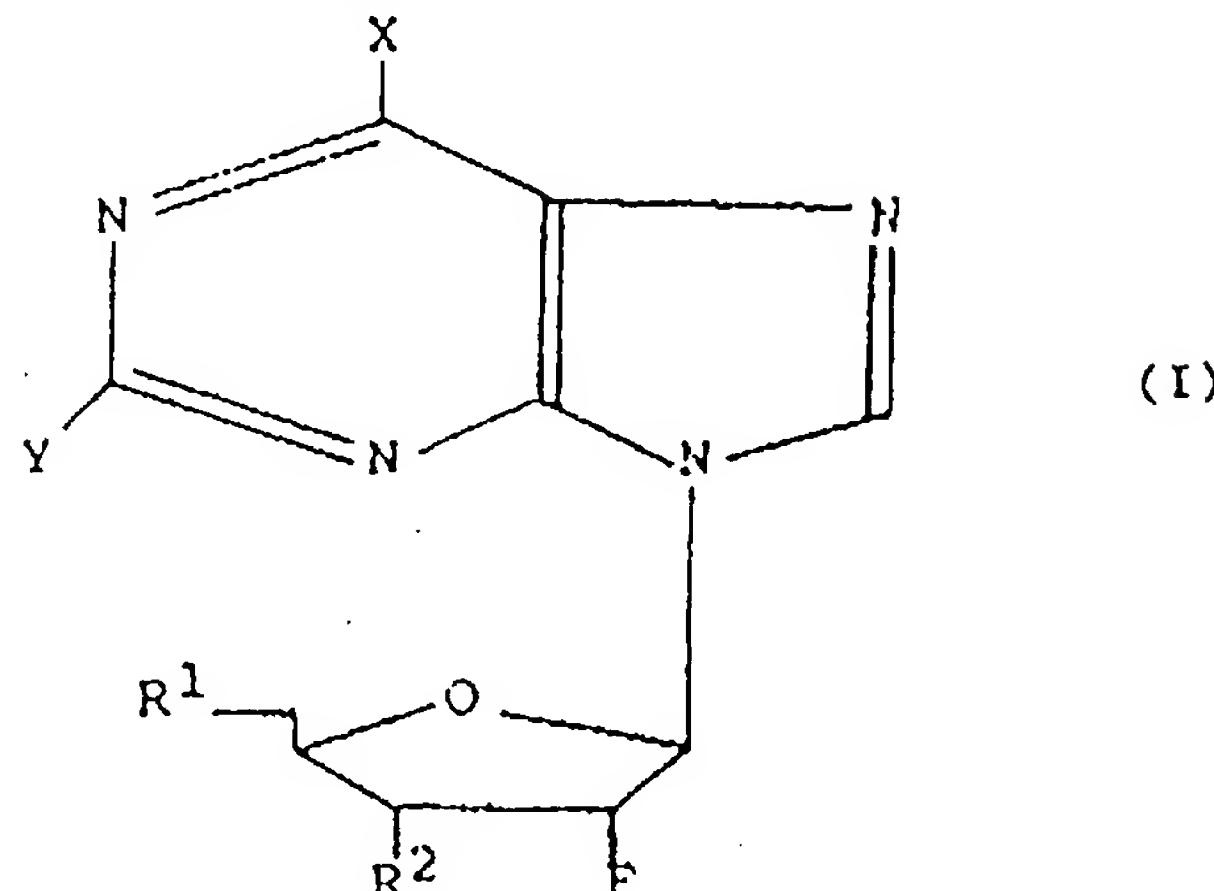
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(ii) faire réagir un composé de formule (I) dans lequel R¹ et/ou R² ne sont pas un groupe hydroxy avec un agent servant à convertir ledit groupe R¹ et/ou R² en un groupe hydroxy.

Revendications pour les Etats contractants suivants : ES, GR

5

1. Procédé de préparation d'un composé de formule (I)

dans laquelle Y est H ou NH₂ ; ;

25 X est un groupe -NR³R⁴ dans lequel R³ et R⁴, qui peuvent être identiques ou différents, représentent chacun un atome d'hydrogène ;

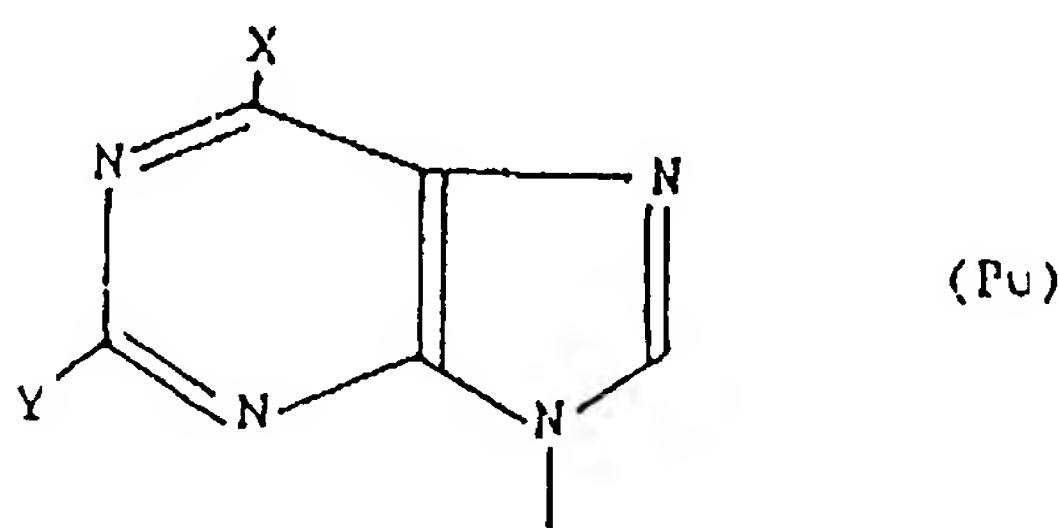
30 un groupe alkyle en C₁₋₆, alkényle en C₂₋₆, cycloalkyle en C₃₋₇, chaque groupe étant éventuellement substitué par un ou plusieurs atomes d'halogène, ou X est un groupe Z-R⁵ dans lequel Z est un atome d'oxygène ou de soufre et R⁵ a la même définition que R³, ou X est un atome d'halogène ou d'hydrogène ; et

35 R¹ et R², qui peuvent être identiques ou différents, représentent chacun :

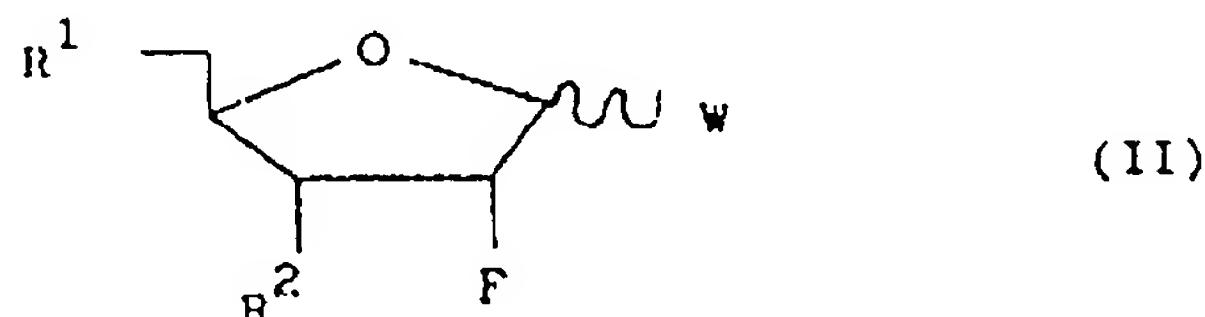
- un groupe hydroxy ;
- un groupe -OCOR⁶H où R⁶ est un groupe bivalent qui est une chaîne linéaire ou ramifiée alkylène en C₁₋₆, alkénylène en C₂₋₆, ou cycloalkylène en C₃₋₇ ; chacune étant éventuellement substituée par un ou plusieurs groupes hydroxy ;
- un groupe -OCO₂R⁷H où R⁷ est une liaison covalente ou est tel que défini pour R⁶ ;
- un groupe -OCOR⁶-COOR⁸ où R⁶ est tel que défini ci-dessus et R⁸ est choisi parmi un atome d'hydrogène, une chaîne linéaire ou ramifiée alkyle en C₁₋₆ ou alkényle en C₁₋₆ chacune étant éventuellement substituée par un ou plusieurs groupes hydroxy ;
- un groupe -OCOR⁷-Z-Ar où R⁷ est tel que défini ci-dessus, Z est soit une liaison covalente soit un atome d'oxygène et Ar est un cycle aromatique non substitué ou substitué par un ou plusieurs atomes d'halogène, un groupe alkyle en C₁₋₆ ou alkoxy en C₁₋₆ ;
- un groupe -OR⁶H où R⁶ est tel que défini ci-dessus ;
- un groupe -OR⁶-Z-Ar où R⁶, Z et Ar sont tels que définis ci-dessus ;
- un groupe -OCOCHR⁹NR¹⁰R¹¹ où R¹⁰ et R¹¹ sont tels que définis pour R⁸, et R⁹ est
 - un atome d'hydrogène,
 - une chaîne linéaire ou ramifiée alkyle en C₁₋₄ éventuellement substituée par un ou plusieurs groupes hydroxy, mercapto, alkoxy en C₁₋₃ ou alkylthio en C₁₋₃

- un groupe R^{12} -A où R^{12} est un groupe alkylène C_{1-4} éventuellement substitué par un ou plusieurs groupes hydroxy et A est un groupe $-COOH$, $-CONH_2$, $-NH_2$ ou $-NH-C(NH)NH_2$ ou A est un système cyclique ou hétérocyclique aromatique ou non aromatique de 4 à 11 éléments contenant 3 à 10 atomes de carbone et 0, 1, 2 ou 3 atomes d'azote dans le cycle, les atomes de carbone et/ou les atomes d'azote du cycle étant éventuellement substitués par un ou plusieurs groupes hydroxy ;
- un groupe $-OCO-R^{13}$ où R^{13} est un hétérocycle de 4 à 7 éléments contenant 3 à 6 atomes de carbone et 0, 1 ou 2 atomes d'azote, les atomes de carbone et/ou les atomes d'azote du cycle étant éventuellement substitués par un ou plusieurs groupes hydroxy ; ou
- un groupe ester mono-, di- ou tri-phosphate ou un sel pharmaceutiquement acceptable de celui-ci autre que des composés de formule (I) dans lesquels R^1 et R^2 sont chacun un groupe hydroxy ou R^1 est un ester en 5' mono-, di- ou tri-phosphate et R^2 est un groupe hydroxy et soit (a) X est OH et Y est NH_2 ou H soit (b) X est NH_2 et Y est H, qui comprend les étapes consistant à soit :

(A) faire réagir une base purique de formule PuH dans laquelle Pu représente le résidu purine :

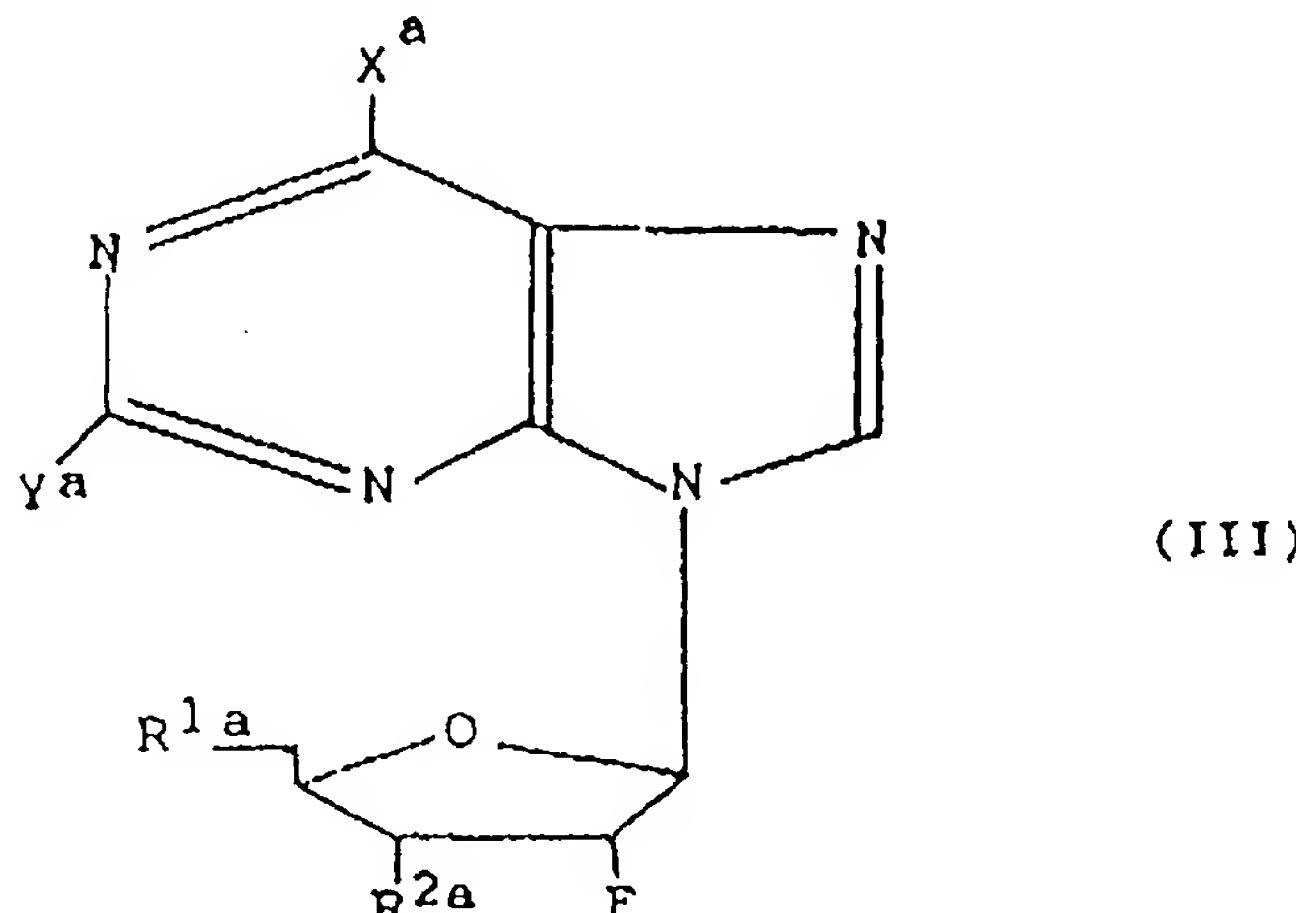


(où X et Y sont tels que définis ci-dessus), ou un sel de celui-ci, avec un composé de formule (II)



dans laquelle R¹ et R² sont tels que définis ci-dessus, et W est soit un ester de phosphate ou un sel de celui-ci soit une partie purine ou pyrimidine (autre que Pu) pour former un composé de formule (I) ;

(B) faire réagir un composé de formule (III)



(dans laquelle X^a , Y^a , R^{1a} et R^{2a} représentent respectivement les groupes X , Y , R^1 et R^2 tels que définis ci-dessus ou une forme précurseur, par exemple protégée, d'un tel groupe fournissant au moins un des groupes X , Y , R^1 et R^2 représenté une forme précurseur) avec un agent servant à convertir

ladite forme précurseur du(des) groupe(s) en le(s) groupe(s) désiré(s) ; et ensuite ou simultanément avec ceci effectuer une ou plusieurs des conversions éventuelles suivantes :

5 (i) faire réagir un composé de formule (I) dans lequel au moins un des groupes R¹ et R² est un groupe hydroxy avec un agent approprié servant à convertir ledit groupe hydroxy en un autre groupe représenté par R¹ et/ou R².

10 (ii) faire réagir un composé de formule (I) dans lequel R¹ et/ou R² ne sont pas un groupe hydroxy avec un agent servant à convertir ledit groupe R¹ et/ou R² en un groupe hydroxy.

15 2. Procédé selon la revendication 1 pour la préparation d'un composé de formule (I) dans lequel R¹ et R² sont soit tous deux OH, soit R¹ est un monophosphate et R² est OH, Y est H ou NH₂ et X est un groupe -NR³R⁴ dans lequel R³ et R⁴, qui peuvent être identiques ou différents, représentent H, un groupe alkyle en C₁₋₆, à condition que R³ et R⁴ ne soient pas tous deux un atome d'hydrogène, ou X est un groupe Z-R⁵ dans lequel Z est O et S et R⁵ est un groupe alkyle en C₁₋₆, ou X est un atome d'halogène ou d'hydrogène, ou un sel pharmaceutiquement acceptable de ceux-ci.

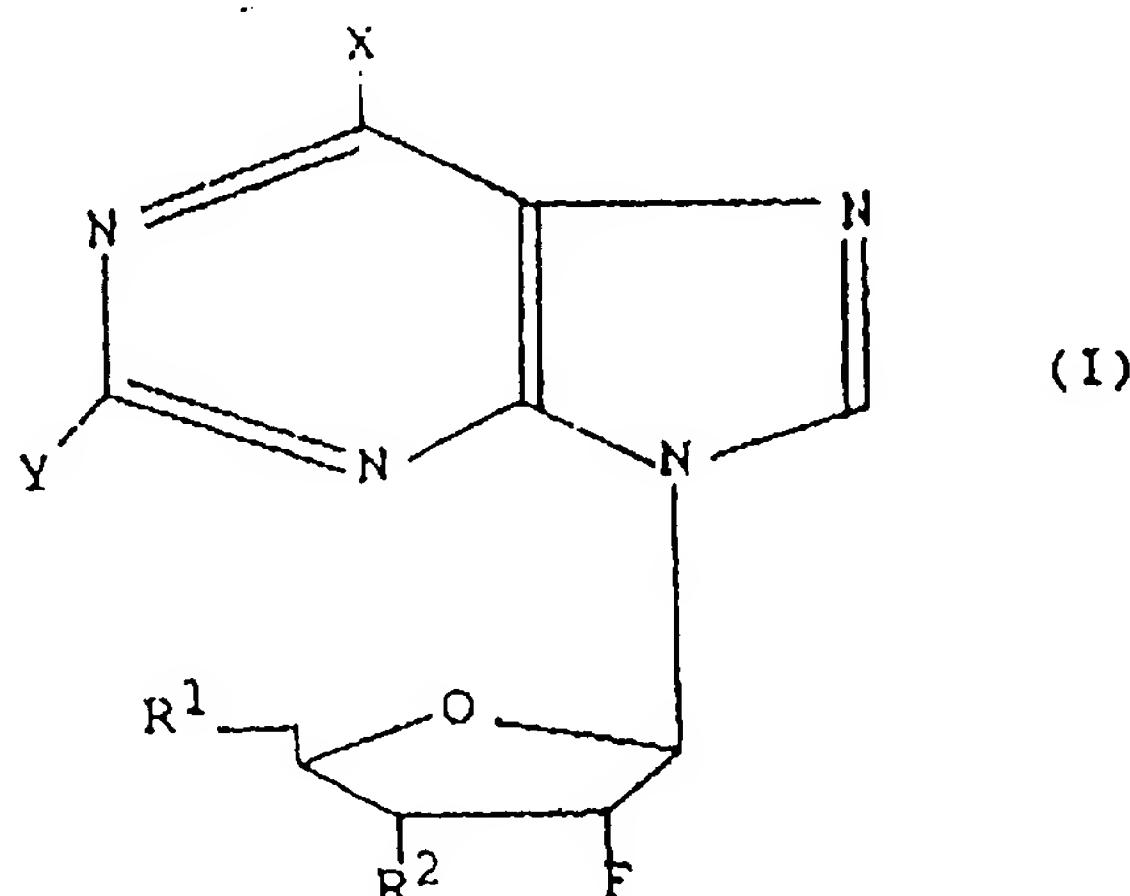
20 3. Procédé selon la revendication 1 ou 2 pour la préparation d'un composé de formule (I) dans lequel R¹ et R² sont tous deux OH, Y est NH₂ et X est H, NH₂ ou Z-R⁵ où Z est O et R⁵ est un groupe alkyle en C₁₋₆, ou un sel pharmaceutiquement acceptable de celui-ci.

25 4. Procédé pour la préparation d'un composé 2,6-diamino-9-(2-désoxy-fluoro-β-D-ribofuranosyl)-9H-purine et des sels pharmaceutiquement acceptables de celui-ci.

30 5. Procédé pour la préparation d'un composé 2-amino-9-(2-désoxy-2-fluoro-β-D-ribofuranosyl)-9H-purine et des sels pharmaceutiquement acceptables de celui-ci.

35 6. Procédé de préparation d'un composé 2-amino-9-(2-désoxy-2-fluoro-β-D-ribofuranosyl)-6-méthoxy-9H-purine et des sels pharmaceutiquement acceptables de celui-ci.

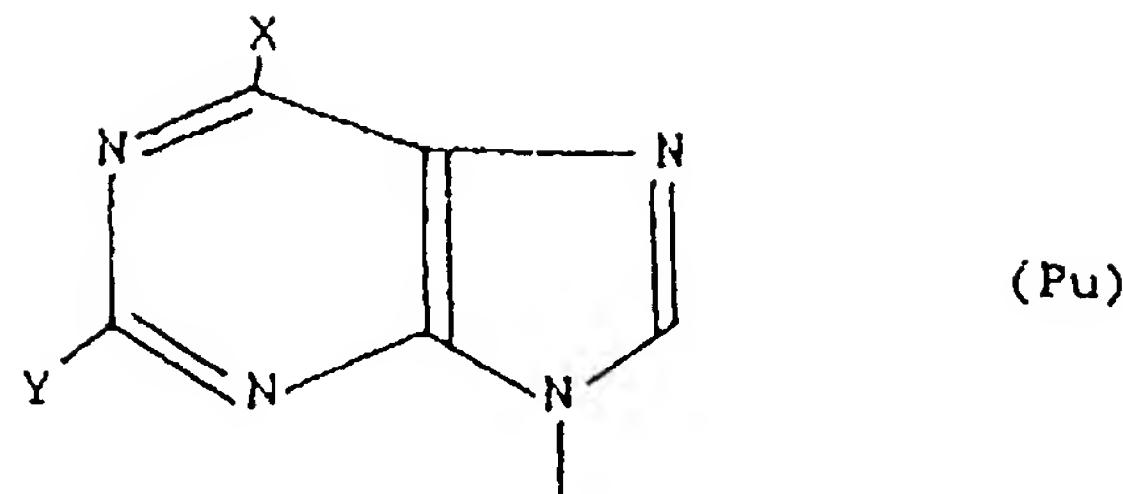
7. Procédé de préparation d'une formulation pharmaceutique contenant un composé de formule (I)



(dans laquelle Y et X sont tels que définis ci-dessus) ou un sel pharmaceutiquement acceptable de celui-ci qui comprend la préparation dudit composé par un procédé qui comprend les étapes consistant à soit :

55 (A) faire réagir une base purique de formule Pu dans laquelle PuH représente le résidu purine :

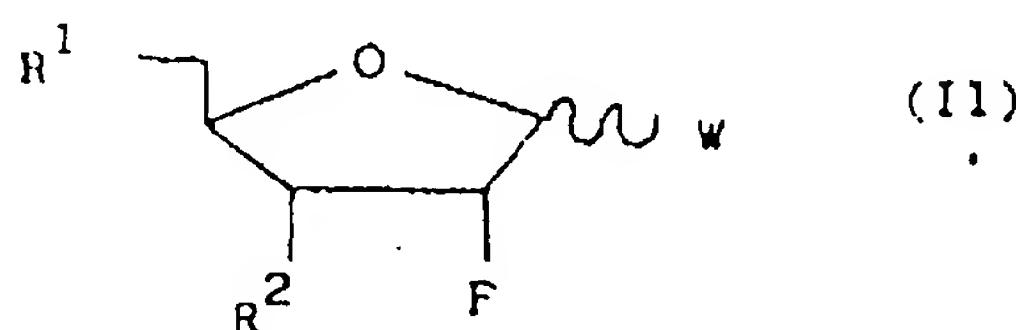
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(où X et Y sont tels que décrits ci-dessus), ou un sel de celui-ci, avec un composé de formule (II)

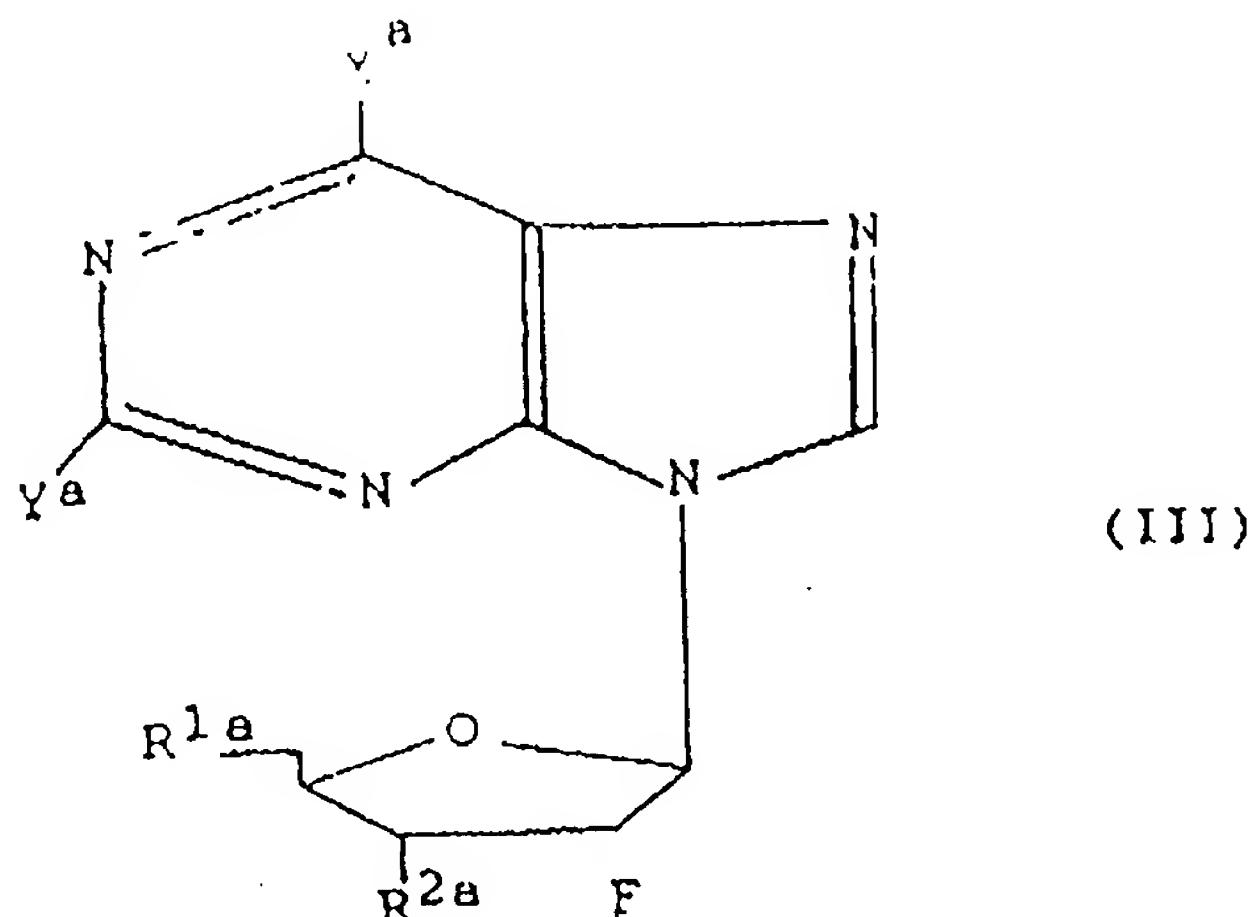
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20 dans laquelle R¹ et R² sont tels que définis ci-dessus, et W est soit un ester phosphate ou un sel de celui-ci soit une partie purine ou pyrimidine (autre que Pu) pour former un composé de formule (I) ;

(B) faire réagir un composé de formule (III)

25



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40 (dans laquelle X^a, Y^a, R^{1a} et R^{2a} représentent respectivement les groupes X, Y, R¹ et R² tels que définis ci-dessus ou une forme précurseur, par exemple protégée, d'un tel groupe fournissant au moins un des groupes X, Y, R¹ et R² représenté une forme précurseur) avec un agent servant à convertir ladite forme précurseur du (des) groupe(s) en le(les) groupe(s) désiré(s) ;

et ensuite ou simultanément avec ceci effectuer une ou plusieurs des conversions éventuelles suivantes :

45

(i) faire réagir un composé de formule (I) dans lequel au moins un des groupes R¹ et R² est un groupe hydroxy avec un agent approprié servant à convertir ledit groupe hydroxy en un autre groupe représenté par R¹ et/ou R².

50 (ii) faire réagir un composé de formule (I) dans lequel R¹ et/ou R² ne sont pas un groupe hydroxy avec un agent servant à convertir ledit groupe R¹ et/ou R² en un groupe hydroxy.

et à réunir le composé résultant de formule (I) avec un ou plusieurs véhicules pharmaceutiquement acceptables pour former ladite formulation pharmaceutique.

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